CHEMISTRY
of
THE CARBOHYDRATES

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BY

WILLIAM WARD PIGMAN

The Institute of Paper Chemistry, Appleton Wisconsin

AND

RUDOLPH MAXIMILIAN GOEPP, JR.

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PREFACE

It is hoped that this monograph will fill the urgent need for an introductory survey of the broad field of the carbohydrates including sugars, derived products, and polysaccharides from organic, physical, analytical, biological, and industrial chemical aspects. In any case, this book brings together much hitherto unassembled material and considers many subjects both from the standpoint of products and of the reactions involved. The latter approach may make this work of interest to organic and physical chemists who are not specialists in the carbohydrate field. In general, the intention has been to write this monograph for the use of research workers and graduate students, but it is hoped that generally the style is such that the book can be used to advantage by readers not having an extensive acquaintance with organic and physical chemistry.

The preparation of a specialized monograph of this type presents many problems for the author; two of the most difficult are the selection of material and the proper balance between various subjects. The number of published researches is so great that only a small part of the material can be presented in a single volume, and it would appear that the complete summation of researches in special fields requires new publication media. For the carbohydrates, it is hoped that the "Advances in Carbohydrate Chemistry" in time will bridge the gap between the publication of original papers and passage of the data and concepts into texts and the general body of scientific knowledge. Certainly there is no conflict of purpose between the present book and the "Advances," for the former represents a brief summary of a broad field, whereas the latter gives detailed discussions of restricted scope.

Numerous subjects of considerable importance such as carbohydrate metabolism, fermentations and photosynthesis have been omitted or covered only briefly. The discussion of cellulose and starch is inadequate in terms of the amount of material available, but it was felt that a book purporting to cover carbohydrates must include something on these important subjects, in any case there are numerous more extensive works on the subject. Perhaps only the author's privilege and the particular interest of the writer can justify the depth of the discussion of several other subjects. Within these limitations, it is hoped that this monograph presents a fair view of the subject of carbohydrates in its broad aspects.

The problem of the proper citation of old work is often very difficult, particularly when reviews are not available or when old work has lost its significance. The citation of original work by the author refers only to the specific statements involved. When a statement may involve work prior to the reference actually given, the word "See" may be inserted before the actual citation. Practically all the references were inspected in the

original; in most of the remaining instances, the secondary references employed are also given.   The literature covered comprises the period to early 1946, but some later work is included.

In spite of extensive attempts to avoid errors, undoubtedly additional ones will be found   It will be greatly appreciated if such instances are called to the attention of the writer

The current status of nomenclature in the carbohydrate field, in spite of the excellent work of the Nomenclature Committee of the Division of Sugar Chemistry and Technology of the American Chemical Society, has created many difficulties in the preparation of this book.   With but few exceptions, the recommendations of this committee have been followed, but the scope of the recommendations is extremely limited.   Attempts have been made to point out difficulties and possible improvements in nomenclature without completely accepting or standardizing the usage in the book.   The most radical changes are in the names of the dibasic or "aric" acids, but even here old and new names are intermingled with the hope of a gradual introduction of the more systematic nomenclature   The preparation of the index required that a definite stand be made on many questions of nomenclature, and, in general, represents the nomenclature favored by the writer   To reduce difficulties in the use of the index, an extensive system of cross entries and multiple headings including synonyms is used.   Undoubtedly, no one, including the writer, will be completely satisfied with the nomenclature, but there seems to be no better solution until progress is made by national or international agreement

Active work on the present book commenced in 1940 while the writer was associated with the National Bureau of Standards and was continued subsequently at the Corn Products Refining Co. and the Institute of Paper Chemistry. Originally, Dr. H. S. Isbell was to participate as co-author. The pressure of other duties prevented active collaboration on his part, but the writer is deeply indebted to Dr Isbell for suggestions of the general organization of material and for the use of his valuable file of reference cards

In 1943, Dr. R Max Goepp, Jr., decided to participate as co-author. Dr Goepp's contribution to the present book is very significant, although his actual written contributions are limited to the Introduction (Chapter I) and the section on anhydrides in Chapter VIII.   His death on October 3, 1946, as a result of an airplane accident while traveling to Germany under the authority of the Office of Technical Services, U S. Department of Commerce, ended a brilliant and promising career and prevented the completion of his assignment as co-author.   His notes were used by Drs. Green and Soltzberg and the present writer in the preparation of Chapters VI and VII.   Dr. Goepp, however, had devoted much time to critical evaluations of the other chapters, and his suggestions were found to be of the utmost value.

Dr. Sol Soltsberg and Dr. John W. Green were mainly responsible for the preparation of Chapters VI and VII, respectively. The present writer is deeply grateful for their help. Dr. Green also undertook the principal responsibility for the preparation of the subject index, an extremely difficult and time-consuming task.

The assistance of many other individuals has been of the greatest importance. Professor C. S. Hudson read many of the chapters and gave many valuable critical comments. His comments, as well as those of persons mentioned hereafter, added much to the present book, but the statements made therein are the sole responsibility of the writer and are not to be blamed on any of the co-operating individuals. Others who have read one or more chapters are:

Drs. R. S. Tipson, H. K. Rutherford, A. M. Sookne, Sol Soltzberg, L. E. Wise, Harriet Frush, D. H. Brauns, H. G. Fletcher, Jr., E. Anderson, N. K. Richtmyer and T. J. Schoch.

The advice and aid with proof reading on the part of Dr. C. J. West were of great help. Drs. Hewitt G. Fletcher, Jr., Sol Soltzberg and Charles J. Pedersen devoted much time to the reading of the page proofs and suggested important changes. Their help is deeply appreciated.

The editorial work in connection with a project of this type is a grueling but essential task. Particular gratitude for help in this connection is due Dr. Mary Grace Blair and Dr. Elizabeth Osman; both revised or wrote several sections and contributed valuable suggestions. The assistance of Miss Marilyn Podest, Mrs. John W. Green and Mrs. W. Bruce Weber is also gratefully acknowledged.

The author index was prepared by Miss Hannah Bergman of the Academic Press. Her help, as well as that of the Academic Press, generally, was of great value. Miss Mary Ann Zastrow and Miss Marilyn Podest cooperated in this work.

Valuable sustaining assistance was provided by Mr. John G. Strange, Mr. Westbrooke Steele and Dr. H. F. Lewis (of the Institute of Paper Chemistry), Drs. E. W. Reid, H. F. Cox, A. L. Elder and S. M. Cantor (of Corn Products Refining Co.), Mr. F. J. Bates (of the National Bureau of Standards), and Mr. K. R. Brown and Dr. R. S. Rose, Jr. (of Atlas Powder Co.). The organizations represented by these individuals provided important encouragement for this project.

To the authors of reviews and books in the carbohydrate field, the present writer is deeply indebted, for these reviews were used extensively in the preparation of this book.

WARD PIGMAN

Appleton, Wisconsin
April 1, 1948

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INTRODUCTION*

1. Development of Carbohydrate Chemistry[1]

The culture of sugar cane and the use of the juices as a sweetening agent appear to have originated in northeastern India. As early as 300 A.D., the crystalline sugar was known and used. Sugar cane culture was extended to China around 100 A.D. and to Egypt around 640 A.D.; from Egypt, the culture and use of the sugar spread gradually over North Africa to Spain and Sicily. The introduction into North America is ascribed to Columbus who brought the plant to Santo Domingo on his second voyage. Sugar cane cannot be grown well in Europe because it requires a tropical or semi-tropical climate, but the sugar was known in Europe during the fourteenth and fifteenth centuries and used as a costly sweetening agent. However, by 1600 many sugar refineries had been erected in Europe, and the use of cane sugar had become widespread.

The necessary restriction of the culture of sugar cane to tropical or semi-tropical lands stimulated the search for sweetening materials which could be obtained from plants native to the temperate region. This search led to the technical development on the European continent of the sugar beet during the latter part of the eighteenth century and especially in the early years of the nineteenth because of the continental blockade during the Napoleonic wars.

The desire to find sweetening agents stimulated the study of known products and of new sources. Honey, grape juice, and raisins were known to contain material which crystallized under some conditions. Marggraf in 1747 described a type of sugar which occurs in raisins. Lowitz (1792) isolated a sugar from honey which he indicated to be different from cane sugar. Proust (1802) claimed that grapes contain a sugar which is different from sucrose. The action of acids on starch was shown to produce a sweet sirup from which a crystalline sugar was isolated by Kirchoff in 1811. Later workers established that the sugar contained in grapes is identical with that in honey, in diabetic urine, and in the acid-hydrolyzates of starch and cellulose; it was given the name of glucose by Dumas (1838) and of dextrose by Kekulé (1866). Emil Fischer revived the name glucose, and it is now used generally in scientific work.

* The principal portion of this chapter was prepared by the late R. Max Goepp, Jr.

[1] For more details of the history and earlier work, the reader is referred to the following references from which the present discussion was abstracted

a. E. O. von Lippmann, "Geschichte des Zuckers", 2nd Ed., Berlin (1929)

b. "Beilsteins Handbuch der organischen Chemie." Vol. 31, J. Springer, Berlin (1938).

Our present knowledge of carbohydrate chemistry is an outgrowth of scientific inquiry into the composition of such common substances as sugar, honey, milk, starch, cotton, wood, vegetable gums, crabshells, and also the less familiar sweet principles, acids, pigments, and pharmacological extractives of numerous plants.

Due to their ease of isolation and purification, sucrose, lactose (milk sugar), starch, cotton cellulose, glucose and fructose were among the first to be studied, and their empirical composition was found to correspond to the general formula $C_n(H_2O)_x$. Since structural chemistry and the existence of hydroxyl groups and hydrogen as structural elements was unknown at the time, the substances were looked upon quite naturally as compounds of carbon and water, and were termed carbohydrates (French, *hydrates de carbone*).

It was soon learned that acid hydrolysis converted starch and cellulose, $[C_6(H_2O)_5]_x$, into glucose, $C_6(H_2O)_6$, with the uptake of one mole of water per C_6 unit. Cane sugar, $C_{12}(H_2O)_{11}$, took up one mole of water to give two $C_6(H_2O)_6$ sugars (hexoses), glucose and fructose. Lactose, another $C_{12}(H_2O)_{11}$ compound, gave glucose and galactose, both $C_6(H_2O)_6$. Hydrolysis of cherry gum yielded arabinose, $C_5(H_2O)_5$, a pentose. Another C_6 sugar, sorbose, was discovered in an old, fermented sample of sorb apple juice. Hence some of the carbohydrates came to be considered as anhydride polymers of the simpler sugars. Further work showed that arabinose, glucose, and galactose were polyhydroxy aldehydes (aldoses) while fructose and sorbose were polyhydroxy ketones (ketoses). Somewhat later a third C_6 aldose (aldohexose), mannose, was synthesized from mannitol, and subsequently found in nature. The actual structure of the three natural C_6 aldoses was unknown, but after the development of the Le Bel-van't Hoff theory it was evident that they were stereoisomers, since all were straight-chain compounds.

Meanwhile, the series of naturally occurring, homologous, straight-chain polyhydric alcohols: glycol, glycerol, erythritol (C_4), arabitol (C_5), mannitol, dulcitol, sorbitol, and iditol (C_6), and persietol (C_7), had been discovered. They had the general formula $C_n(H_2O)_nH_2$, (in modern terms, $HOCH_2(CHOH)_nCH_2OH$). Erythritol and the higher members were crystalline, sweet-tasting and water-soluble. The four hexitols were known to be isomeric, but their relationship to each other and to the five natural C_6 sugars was not known until Emil Fischer's classical work in the early nineties.

Three dibasic acids of the series $HOOC(CHOH)_nCOOH$ were likewise discovered very early, the C_4 tartaric acid from wine lees, and the isomeric C_6 mucic and saccharic acids from the nitric acid oxidation of lactose and of cane sugar.

Similarly, a few monobasic or aldonic acids corresponding to the oxida-

tion of one end of a polyhydric alcohol or the aldehyde group of a sugar—hence of the formula $HOCH_2(CHOH)_{n-2}COOH$ —were found. The first was glyceric acid, from glycerol and nitric acid, the next gluconic acid, from glucose. Subsequently gluconic acid was found to be a common product of the bacterial oxidation of glucose.

Application of established organic chemical reactions to these compounds demonstrated that the simple sugars, as aldehydes or ketones, could be reduced to alcohols, oxidized to mono- or dibasic acids, and reacted, under suitable conditions, with carbonyl reagents, such as phenylhydrazine, hydroxylamine, and hydrocyanic acid. The hydrocyanic addition reaction led to Kiliani's proof of a 2-keto structure for fructose, and also allowed the conversion of a C_5 aldehyde sugar to a C_6 sugar acid. Fischer showed that phenylhydrazine could be used for isolating various sugars, and that glucose, mannose and fructose had the same stereochemical configuration on carbons 3, 4 and 5. Similarly the alcohols could be oxidized to mixtures of sugars and sugar acids by nitric acid, and the acids, in the form of their lactones, could be reduced to the aldehyde sugars, and ultimately to the alcohols, by sodium amalgam.

$$
\begin{array}{ccccccc}
CH_2OH & & CHO & & COOH & & COOH \\
| & & | & & | & & | \\
(CHOH)_{n-2} & \longleftarrow & (CHOH)_{n-2} & \rightleftarrows & (CHOH)_{n-2} & \longrightarrow & (CHOH)_{n-2} \\
| & & | & & | & & | \\
CH_2OH & & CH_2OH & & CH_2OH & & COOH \\
\text{Polyhydric} & & \text{Sugar} & & \text{Aldonic} & & \text{Saccharic} \\
\text{alcohol} & & & & \text{acid} & & \text{acid}
\end{array}
$$
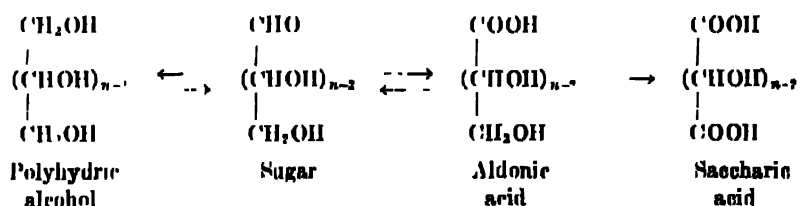
These interconversions, applied to the naturally occurring sugars, sugar alcohols, and derived acids, played an essential part in elucidating their structure, so that the chemistry of all four groups had a parallel development. The following family relationships were discovered.

Dulcitol ⟷ galactose ⟷ galactonic acid ⟶ mucic acid

Mannitol ⟷ mannose ⟷ mannonic acid ⟷ *manno*-saccharic acid

fructose

Sorbitol ⟷ glucose ⟷ gluconic acid ⟶ *gluco*-saccharic acid

sorbose

Iditol ⟵--- idose ⟷ idonic acid -⟶ *ido*-saccharic acid
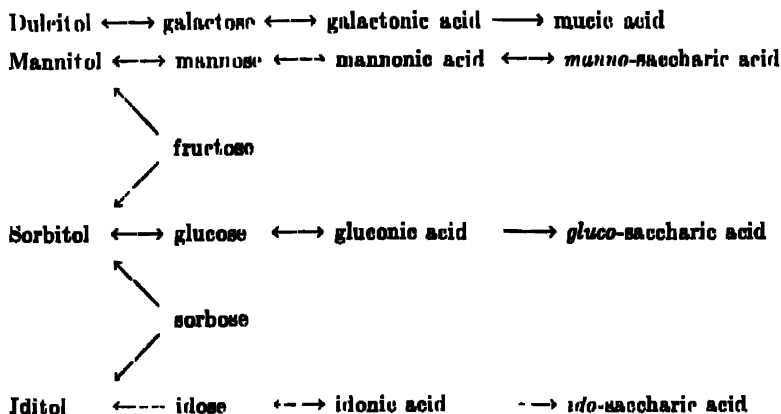
It is noteworthy that sorbitol, glucose, and gluconic acid are all readily fermented, or utilized as a carbon source, by numerous species of molds and bacteria.

The sodium amalgam reduction of a saccharic acid monolactone to an aldonic acid goes by way of an intermediate, aldehyde acid. In this way glucuronic acid was first synthesized from a *gluco*-saccharic monolactone acid. It had been discovered previously in urine, hence the name.

$$
\begin{array}{ccc}
\text{COOH} & & \text{CHO} \\
| & & | \\
\text{(CHOH)}_4 & \rightarrow & \text{(CHOH)}_4 \\
| & & | \\
\text{COOH} & & \text{COOH} \\
\text{D-}gluco\text{-Saccharic} & & \text{D-Glucuronic acid} \\
\text{acid (as monolactone)} & &
\end{array}
$$
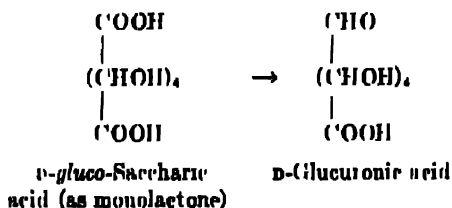
Somewhat later, it was found that the C_6 aldehyde acids, or uronic acids, are widely distributed (citrus pulp, beet pulp, seaweed, cartilage, mucin, agar-agar) as polymers, known as polyuronides, analogous to starch and cellulose. Here again the glucuronic, mannuronic, and galacturonic acids were the only representatives found in nature.

Glucose, mannose and galactose residues likewise appear in epirhamnose, rhamnose, and fucose, six-carbon sugars in which the hydroxyl on carbon 6 is replaced by hydrogen, giving the 6-desoxy sugars or methyloses.

Two other natural sugar types have been found, the 2-desoxy sugars, represented by the C_5 sugar, 2-desoxyribose, $HOCH_2(CHOH)_2CH_2CHO$, present in many cell nuclei, and the 2-amino sugar, glucosamine, $HOCH_2$-$(CHOH)_3CHNH_2CHO$, obtained by the acid hydrolysis of chitin, a structural polymer in the shells of insects and crustaceans. Neither of these biologically important sugars has the empirical composition $C_6(H_2O)_6$.

The glycamines, 1-desoxy-1-amino glykitols $CH_2OH(CHOH)_nCH_2NH_2$, are another class of straight chain, polyhydroxylic compounds, wherein the characteristic functional group is NH_2. They do not occur naturally, but ribamine, $HOCH_2(CHOH)_3CH_2NH_2$ is a starting material for one of the riboflavin syntheses. The glycamines and their N-substituted derivatives are obtainable by pressure hydrogenation of sugars in the presence of ammonia, primary, or secondary amines.

A series of naturally occurring compounds having the carbohydrate formula $C_6(H_2O)_6$ is known, which are not sugars, but cyclic polyhydric alcohols, or cyclitols, $C_6H_6(OH)_6$, of which meso-inositol, possibly a provitamin, is the most important.

The simple carbohydrate formula $C_n(H_2O)_n$ is also shared by a class of saccharinic acids, $C_{n-1}H_{n+1}(OH)_{n-2}COOH$, obtainable by the alkaline isom-

erization of the sugars. The two lowest members, acetic and lactic acid, are the important natural representatives.

Ascorbic acid, 2 keto-L-gulonic lactone, (Vitamin C), (enol form),

$$(HO)H_2C—C—C—C=C—CO$$
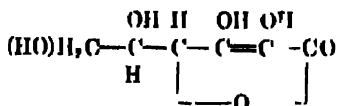
is synthesized industrially from sorbitol by way of sorbose, and several isomers of lesser antiscorbutic activity have also been made by comparable procedures.

Carbohydrate chemistry includes the chemistry of these homologous, or isomeric, series of polyhydroxy compounds, and their derivatives. Today, the term carbohydrate is frequently applied to the unsubstituted members of any of the biologically important series of polyhydroxy compounds, and particularly the naturally occurring compounds. At the same time, it should be noted that the narrower, historical definition of carbohydrates as the sugars or their polymers, namely, the saccharides, is still frequently employed, particularly in fields outside of biochemistry.

2. General Chemistry

The carbohydrate compounds $HOCH_2(CHOH)_nX$ differ from the paraffinic alcohols, aldehydes, ketones, acids, amines and ethers in several important respects.

A. Stereoisomerism. *The repeating unit, CHOH, contains an asymmetric carbon, in contrast to the CH_2 of the paraffinic series.* As these accumulate, a large number of stereoisomers become possible. For series with unlike terminal groups, such as the *aldoses, aldonic acids,* and *alduronic acids,* the number of stereoisomers is 2^n, where n is the number of asymmetric carbon atoms. For like-ended compounds, such as the polyhydric alcohols, and the dibasic acids, the number of isomers is less, due to the greater symmetry. Thus, in the carbohydrates, the typical isomerism involves the H, OH space arrangement, while in the paraffin series, isomerism is principally that of chain branching. A few branched-chain sugars are known, however.

Although almost all of the theoretically possible sugar alcohols, sugars, mono- and di-basic acids are known up through C_6, only a few in each group occur in nature, as Table I shows.

The naturally occurring carbohydrates have been studied much more than the synthetic members. Of the simple sugars (monosaccharides), glucose is by far the commonest sugar, with xylose probably next in rank.

B. Activation by Carbonyl Groups. *The carbonyl groups in the aldoses and ketoses have the usual activating effect on the adjacent C-linked or α-hydrogens, so that enolization may be evoked by inorganic or organic bases*

These enolizations are shown for an aldose or ketose (a-d).

$$
\begin{array}{ccccc}
\text{HCO} & & \text{HCOH} & & \text{HCO} \\
| & \rightleftharpoons & \| & \rightarrow & | \\
\text{HCOH} & & \text{COH} & \leftarrow & \text{HOCH} \\
| & & | & & | \\
\text{(a)} & & \text{(b)} & & \text{(c)}
\end{array}
$$

$$
\begin{array}{c}
\downarrow\uparrow \\
\text{CH}_2\text{OH} \\
| \\
\text{CO} \\
\\
\text{(d)}
\end{array}
$$
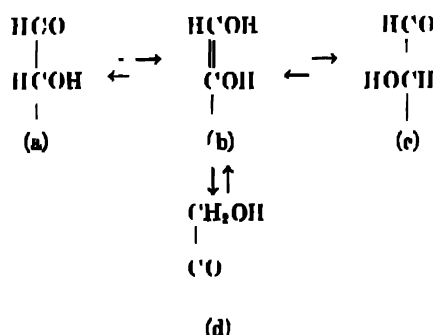
When, as is usually the case, the migrating hydrogen is attached to an asymmetric, hydroxyl-bearing carbon, a hypothetical enediol (b) is postu-

TABLE I

Possible and Naturally Occurring Carbohydrates Exclusive of Racemic and Anomeric Forms

No. of Carbons	3		4		5		6		7		Total	
	Pos-sible	Found	Pos-sible	Found	Pos-sible	Found	Pos-sible	Found	Pos-sible	Found	Pos-sible	Found
Alcohols	1	1	3	1	1	2	10	4	16	2	31	10
Aldoses	2	1	1	0	8	1	16	4	32	2	62	11
2-Ketoses	1	1	2	0	1	1	8	2	16	1	31	5
Aldonic Acids	2	1	1	0	8	0	•16	1	32	0	62	2
Alduronic Acids	2	0	4	0	8	0	16	3	32	0	62	3
Dibasic Acids	1	0	3	1	4	0	10	0	16	0	34	1

* The figures for the number of naturally occurring isomers are only approximate since confirmation is lacking for some reported occurrences.

lated, in which the asymmetry at carbon 2 has been destroyed. Hence reversion of the enediol to the hydroxycarbonyl forms (a), (c), and (d) allows inversion of the H—OH configuration on the second carbon. Where the second carbon is the only asymmetric carbon present, as in glyceraldehyde,

$$
\begin{array}{c}
\text{CHO} \\
| \\
\text{HCOH} \\
| \\
\text{CH}_2\text{OH}
\end{array}
$$
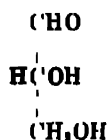
this inversion produces a racemization of the active forms. Where more than one asymmetric carbon is present, a new stereoisomer is formed, e.g., mannose from glucose.

The change of configuration at the second carbon—known as 2-epimerization—is one of the important routes to the synthetic sugars, particularly for the aldonic acids which undergo a similar change in the presence of nitrogenous bases such as pyridine.

Instead of reverting to the hydroxy aldehyde, the hypothetical enediol (b) may pass to the hydroxy ketone (d), which, in the sugar series, means shifting from aldose to ketose, e.g., glucose to fructose. Thus glucose, mannose and fructose have a common enediol (although it has never been isolated), so that, in the presence of mild aqueous alkali, these three are interconvertible, as Lobry de Bruyn and Alberda van Ekenstein showed. A true equilibrium is not set up, however, due to side reactions.

This aldose-ketose shift is, in effect, a migration of the carbonyl group from carbon 1 to carbon 2. Theory would predict further shifting from carbon 2 all the way to carbon 6. Actually, no 3-ketohexose has been isolated, but sorbose has been obtained from D-galactose (a 3-epimerization) and the identification of allitol among the products of the alkaline reduction of glucose is further evidence for epimerization at carbon 3 and for the formation of a 2,3-enediol of glucose:

$$
\begin{array}{ccccc}
\text{CHO} & & \text{HCOH} & & \text{CH}_2\text{OH} \\
| & & \parallel & & | \\
\text{HCOH} & & \text{COH} & & \text{CO} \\
| & & | & & | \\
\text{HOCH} & \rightarrow & \text{HOCH} & \rightarrow & \text{HOCH} \\
| & & | & & | \\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} \\
| & & | & & | \\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} \\
| & & | & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
\text{Glucose} & & \text{(1,2-Enediol)} & & \text{Fructose}
\end{array}
$$

$$
\begin{array}{ccccc}
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
| & & | & & | \\
\text{COH} & & \text{HCOH} & & \text{HCOH} \\
\parallel & & | & & | \\
\text{HOC} & \rightarrow & \text{CO} & \xrightarrow{\text{H}_2} & \text{HCOH} \\
| & & | & & | \\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} \\
| & & | & & | \\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} \\
| & & | & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
\text{(2,3-Enediol)} & & \text{(3-Keto hexose)} & & \text{Allitol}
\end{array}
$$
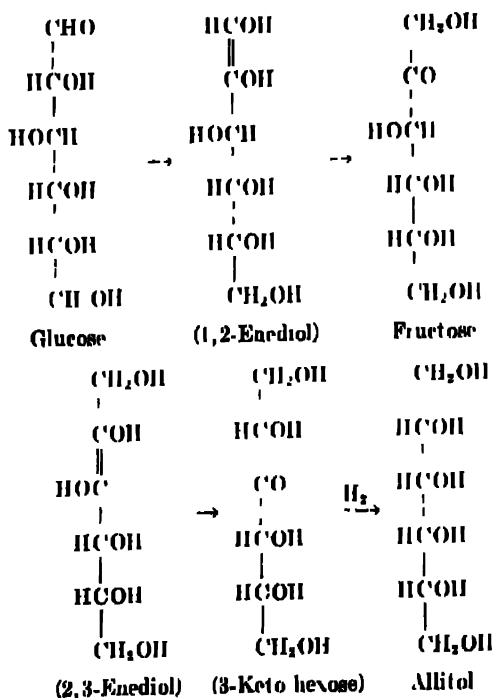
Glyceraldehyde and its related ketose, dihydroxyacetone, can likewise undergo external aldol condensation to yield a mixture of DL-fructose and

five tautomeric forms of the same compound. From glucose, only the α- and β-pyranoses can be obtained as such; the other modifications cannot be crystallized from solution, and are known only as derivatives. The equilibrium, if catalyzed, is established almost instantly; otherwise several hours or even days may be required.

Thus an aqueous solution of glucose and fructose, such as is encountered frequently in natural products, is a complex mixture of at least ten tautomeric substances, all giving the same phenylosazone with phenylhydrazine. Without such a simplifying reagent, the original investigation of these protean structures would have been almost impossible.

In the lactones, the absence of α,β isomerism restricts the tautomers to the free acid, and the gamma and delta lactones, all three of which can be crystallized from solution, in many cases. Equilibrium is reached much more slowly than with the sugars. Oddly enough, the 1,4 or gamma lactones are the more stable, while the 1,5 or pyranose sugars are the favored form.

Since the optical rotations of the various tautomeric forms of sugars and lactones vary widely, the rotation of the mixture in solution approaching equilibrium will change with time either up or down from the initial value. This is called mutarotation. For some sugars, its origin may be very complex.

The polyhydric alcohol inner anhydrides IV, are formed only under conditions approximating those required for the etherification of aliphatic alcohols. However, these conditions exist during the commercial esterification of polyhydric alcohols. Complex mixtures of esterified mono- and di-anhydrides are therefore obtained from the direct high temperature esterification of sorbitol or mannitol. Reopening of the anhydro rings is not readily accomplished, so that this inner etherification is not considered reversible, and there is no mutarotation. Numerous position isomers are possible; sorbitol gives three furanoid and two pyranoid monoanhydrides - or sorbitans while three dianhydrides of mannitol are known. The 1,5-anhydrides of sorbitol and mannitol occur naturally, but rarely, as polygalitol and styracitol. Glycerol and ethylene glycol can give both inner and external ethers under similar conditions.

Once the potentially ring-forming hydroxyl groups at carbon 4 or 5 or sometimes 6 in the sugars or aldonic acids are substituted as by acetyl or methyl groups, the compounds behave like ordinary aliphatic aldehydes or acids. This is usually accomplished by fixing the carbonyl group in a diethyl mercaptal, and regenerating after the hydroxyls have been substituted. Similarly, esterification of the sugar acids by external hydroxy compounds allows the lactonizable stem hydroxyls to be reacted.

D. The Glycosidic Hydroxyl and Polymeric Carbohydrates. The ring modifications of the sugars and uronic acids all have a characteristic feature

—the hemiacetal hydroxyl group formed at the original carbonyl by the lactol ring

R = H, CH₂OH
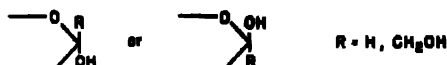
and known as the hemiacetal, anomeric or glycosidic hydroxyl. It resembles a tertiary aliphatic hydroxyl group in its ease of reaction with alcohols to form full acetals which when external are known as glycosides. This reaction, which is catalyzed by acids or enzymes, allows the selective etherification (actually acetalization) of sugars at this position. The methyl and ethyl glycosides are obtainable merely by refluxing the sugar with excess of the alcohol, using hydrochloric acid or sulfuric acid as catalyst.

When stabilized by glycoside formation, α- and β-anomers of the same parent sugar can frequently be isolated, whereas isolation of both of the free alpha and beta ring forms from the equilibrium mixture of various forms in solution has been realized for relatively few sugars.

Favorable configuration of the other ring hydroxyls may allow internal glycosidation to the inner full acetal, or glycosan.

β-Glucose β Glucosan
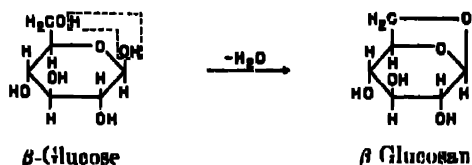
The glycosidic hydroxyl may be esterified, but not much more readily than the primary and certain secondary hydroxyl groups, so that hemiacetal monoesters are not readily obtainable directly by partial acylation of the free sugars. Once formed, however, the 1-acetoxy group of glucose pentaacetate is *selectively* replaced by a halogen of a halogen acid to give tetraacetyl-α-glucosyl halide. The halogen, usually bromine, is labile, and the compound is a very useful intermediate for the synthesis of glycosides and glycosidic esters. Corresponding compounds of the *aldehydo* and *keto* sugars are known.

The phosphate esters of glucose, fructose, and glycerol are extremely important in the metabolism of sugars by plants or animals. The phosphate groups are attached either to the glycosidic hydroxyl, or to the primary hydroxyl at carbon 6. Benzoylated sugars also occur naturally in certain glycosides.

The naturally occurring oligo- and poly-saccharides, such as sucrose, lactose, starch, cellulose, and the like, are condensation polymers, held

together by glucosidic linkages. They may be largely homogeneous with respect to sugar and linkage, such as cellulose, or composed of various sequences of sugars, uronic acids, or glycosamines, as in the plant gums, cartilage, mucin, and other more complex polysaccharides.

Cellulose consists *essentially* of long chains of glucose molecules, linked at carbon 4 through a β-glycosidic union. The β-D-configuration, in this case *trans* to the hydroxyl at carbon 4, allows an essentially linear polymer of high regularity and hence crystallinity to be built up, where x may reach several thousand.
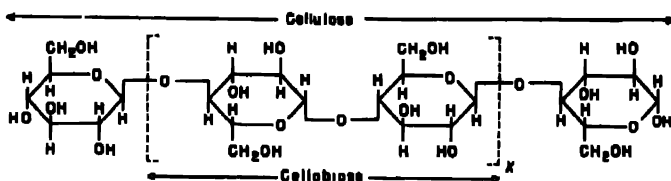
The building unit of this chain is actually cellobiose, a β-linked disaccharide of two glucopyranoses linked 1,4'.

Similarly, the starches are made up of glucopyranose residues linked mainly 1,4', but with α-D-linkages. The succession of α-D-linkages may give the chain a spiral twist as opposed to the linearity of the cellulose chain. The malt enzymes break starches down to give considerable quantities of the disaccharide maltose, which is 4-β-glucose α-glucopyranoside. An important difference from cellulose is that the material is structurally heterogeneous and some of the side chains are attached through the hydroxyls at carbon 6.

Lactose is the 4-β-D-galactopyranoside of glucose connected at carbon 4

In sucrose, however, which has not yet been synthesized chemically, an α-D-glucopyranose and β-D-fructofuranose are linked through the glycosidic hydroxyls of both. In α,α'-trehalose, a rare disaccharide found in the capsules of tubercle bacilli and in certain fungi, two α-D-glucopyranoses are joined through their glycosidic hydroxyls.

Sucrose   α,α'-Trehalose
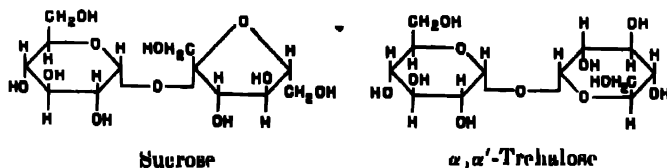
Since rearrangement of these full acetals to an aldehydo or keto form is not possible, these disaccharides do not show the reducing behavior characteristic of the monosaccharides and are called nonreducing disaccharides.

In other naturally occurring polysaccharides the linkages between the sugar molecules is not 1,4', but 1,6'; 1,2'; 1,3' or other combinations.

The disaccharides, and other lower polymers of one or more simple sugars, containing 2 to 10 monosaccharide residues, are termed oligosaccharides. As a class they are definite compounds whose homogeneity is established in most cases and whose structures are largely known. The higher polysaccharides, however, such as starch and cellulose with molecular weights in the thousands and containing hundreds of sugar residues are not chemical individuals in the usual sense. They may be mixtures of very similar polymers having the same or different structural patterns and differing in chain length.

The uronic acid components of the polyuronides are likewise joined glycosidically by 1,4'-linkages. The carboxyl is usually substituted as by a calcium atom or methyl group in pectin, a polygalacturonide. Hydrogenation of reducing disaccharides such as lactose or maltose gives the corresponding glycosyl polyhydric alcohols lactitol and maltitol.

Numerous natural products consist of a sugar usually glucose glycosidically linked with a noncarbohydrate portion known as the aglycon. The aglycon may be a complex phenol, as in the cardiac glycosides of digitalis or in the anthocyanin plant pigments, or it may be some other hydroxylic compound. These naturally occurring glycosides, which may be either α or β, are split by the appropriate enzyme, α- or β-glycosidase. Glucuronic acid is likewise used by mammals as a detoxifying agent by binding an active chemical through the glycosidic hydroxyl and being secreted as the soluble glucuronide

3. Nomenclature and Definitions[2]

A. Scope and General Definitions. The preceding introduction has shown how that part of organic chemistry called carbohydrate chemistry is concerned with several related homologous series, characterized by a plurality of hydroxyl groups, and one or more functional groups. The full-fledged carbohydrates are the five, six and higher carbon members. As the homologous series are descended, the carbohydrate characteristics degenerate, until the atypical first and second members, the sturdy individualists of aliphatic chemistry, are reached.

In Table II the major homologous series are defined, together with synonyms, type endings, and characteristic groupings. Table III lists the 4-, 3- and 2-carbon representatives of the principal series. Compounds of either industrial or biochemical importance are italicized. It is evident that the important 4-carbon compounds are much fewer than the 3- and 2-carbon representatives and that optical activity does not extend below three

TABLE II

Some Important Carbohydrate Types

Type	Description or Synonym	Type Ending	Characteristic Grouping
Polyhydric Alcohol	Sugar Alcohol (Glycitol)	itol	HOH_2C CH_2OH
Polyhydric Alcohol Anhydride	Inner Ether (Glykitan or Glykide)	itan (mono) ide (di)	—O
Desoxy Polyhydric Alcohol	Desitol	desitol	CH_2 , $-CH_2$
Aldose	Monosaccharide Glycose	ose	CHO, O H \\ / C / \\ C OH
Ketose	Monosaccharide Glycose	ose ulose	CO O CH_2OH / C \\ C OH
Glycoside	Full Mixed Acetal	oside	O H \\ / C $-C$ OR
Glycosan	Glycose Inner Full Acetal	osan	—O H \\ / C / \\ —C O -
Glycal	1,2 Unsaturated Glycose (two OH groups lost)	al	—O H \\ / C / —C \\ H
Glycoseen	Ketose Enol (usually enol ester)	-oseen	—O H \\ / C / —C \\ OH COH ‖ CH_3

TABLE II—*Continued*

Type	Description or Synonym	Type Ending	Characteristic Grouping
Osone	2 Keto Aldose	osone	CO CHO
Desose	Desoxy sugar	-desose	$-CH_2-$ $-CH$
Glycosamine	Amino sugar	osamine	$-CHNH_2$ CHO
Glycosylamine	N Glycoside	-sylamine	
Glycamine	1 Desoxy 1 amino glykitol	amine	CH_2NH
Aldonic Acid	Carbohydrate or Sugar Acid	onic acid	COOH
Dibasic Sugar Acid	Aric Acid	aric	HOOC COOH
Uronic Acid	Aldehyde Aldonic Acid	-uronic acid	HOOC CHO
Keto Aldonic Acid		keto onic acid	HOOC CO CH.OH
Saccharinic Acid	Desoxy Aldonic Acid	saccharinic	CH_3 CH , or branched chain

carbons Since ethanol and acetic acid are only monofunctional, their in-clusion in the table is justifiable only in view of their biochemical origin. Acetaldehyde is effectively difunctional, and its linear condensation polymers, including polyvinyl alcohol, are polydesoxy aldoses.

$$CH_2 \quad CH \quad \left(CH_2 \quad CH \right) - CH_2 - CH- \quad CH_2CHO$$
$$OH \qquad \left(\qquad OH \right)_x \qquad OH$$

Formaldehyde has traditionally been classed with the carbohydrates as a monose. Since both formaldehyde and acetaldehyde yield polymers of carbohydrate character, it is not unreasonable to include the condensation interpolymers of formaldehyde such as pentaerythritol, dipentaerythritol, and other branched chain methylols

The α-amino acids of the proteins and of the carbohydrates converge at the 3-carbon series in serine.

The present text will be concerned primarily with the characteristic mono- and polysaccharides and their derivatives, but the important analogies between the behavior of the two- and three-carbon polyfunctional prototypes, and the five- and six-carbon representatives must not be overlooked.

polymerization of formaldehyde); glycolic aldehyde and glyceraldehyde. Glycerose is a mixture of glyceraldehyde and dihydroxyacetone. The single keto tetrose is erythrulose, and the ketopentoses are named from the related pentose as arabinulose or ribulose, and xylulose. All the keto hexoses have trivial names.

B. Configurational and Trivial Names. Thanks mainly to Emil Fischer and his predecessors, trivial names are available for all possible sugars containing three to six carbon atoms, hence for their derivatives. Since the configuration resides in the inner asymmetric carbon atoms, the sugar names provide prefixes for asymmetric carbon sequences of one to four carbon atoms, such as $CHOH$, $CHOR$, $CHNH_2$. They are shown in Table IV, together with the configurations. The characteristic or principal functional group, *e.g.*, CHO in aldose, and $COOH$ in aldonic acids, is at Y. Each prefix has D and L forms, the D-form being shown. The prefix form can be used independently in italics, to show configuration in an asymmetric sequence.

These configurational prefixes are used with generalized names to specify a compound of specific configuration and they often, but not always, indicate the number of carbon atoms in the compound. Thus, the compound obtained by the oxidation of D-glucose with nitric acid is D-*gluco*-saccharic acid, commonly termed merely saccharic acid.

$$HOOC-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}}-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-COOH$$

D-*gluco*-Saccharic acid
(saccharic acid)
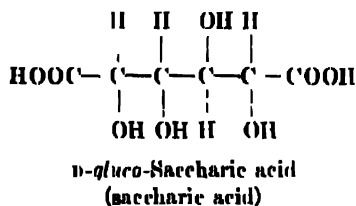
Similar prefixes are used for naming ketoses not having trivial names. This usage is discussed in Chapter II.

The names of several long-known carbohydrates were established before configurational relationships were known, so that the terminology of the commoner carbohydrates, given up to the hexoses in Table V, is not consistent.

Erythritol, adonitol, dulcitol, mannitol and *sorbitol* are originally trivial names applied to naturally occurring alcohols. Mannitol and sorbitol are optically active, adonitol and dulcitol are meso compounds. Erythritol is preferably used only for the *meso* tetritol, the active forms being D- and L-*threitol*, following Hockett.

Sorbitol and sorbose were named independently from the same botanical source, the sorb apple.

The 6-desoxy hexoses are called methyloses, after Votoček. Terms such as "mannomethylose," "galactomethylose" and "glucomethylose" have

TABLE IV

Configurational Prefixes

Carbons	Configuration and Name*

1

```
      H
      |
X —— C — Y
      |
     OH
```
D-*glycero*

2

```
    H   H                  H   OH
    |   |                  |   |
X — C — C — Y          X — C — C — Y
    |   |                  |   |
   HO  OH                 OH   H
```
D-*erythro*   D-*threo*

3

```
    H   H   OH            H   H   H            H   OH  H
    |   |   |             |   |   |            |   |   |
X   C   C   C   Y      X — C — C — C — Y     X   C   C   C — Y
    |   |   |             |   |   |            |   |   |
   OH  OH   H            OH  OH  OH           OH   H  OH
```
D *arabino* (a *abo*)   D *ribo*   D *xylo*

```
    H   OH  OH
    |   |   |
| X  C — C — C — Y
    |   |   |
   OH   H   H
```
D *lyx*

4

```
    H   H   OH  H           H   H   OH  OH          H   OH  OH  H
    |   |   |   |           |   |   |   |           |   |   |   |
X   C   C   C   C   Y    X   C — C   C — C   Y    X   C   C   C   C   Y
    |   |   |   |           |   |   |   |           |   |   |   |
   OH  OH   H   OH         OH  OH   H   H          OH   H   H   OH
```
D *gluc*   D *manno*   D *galacto* (*gala*)

```
    H   OH  H   OH          H   OH  H   H           H   H   H   OH
    |   |   |   |           |   |   |   |           |   |   |   |
| X  C   C   C   C   Y   X   C   C   C   C   Y    X   C   C   C   C   Y
    |   |   |   |           |   |   |   |           |   |   |   |
   OH   H   OH  H          OH   H   OH  OH         OH  OH  OH   H
```
D *ido*   D *gulo*   D *allo*

```
    H   OH  OH  OH          H   H   H   H
    |   |   |   |           |   |   |   |
X   C   C — C   C   Y    X   C — C — C   C — Y
    |   |   |   |           |   |   |   |
   OH   H   H   H          OH  OH  OH  OH
```
D *talo*   D *allo*

* The group Y is the main functional group such as CHO or COOH. Group Y is written at the top when the carbon chain is vertical. (X and Y cannot be hydrogen.)

19

TABLE V

Names of Related Carbohydrates of Three to Six Carbons

Aldose (Also general form for Aldonic Acid, Glycamine, etc)	Glykitol	Dibasic or Aric Acids	
		Common Name	Suggested Aric Form
D- or L-Glyceralde hyde	Glycerol	Tartronic Acid	
		Tartaric Acids	**Tetraric Acids**
D- or L-Erythrose	Erythritol	*meso*-Tartaric Acid	Erythraric Acid
D or L-Threose	D- or L-Threitol or *l* or *d*-Erythritol	D- or L-Tartaric Acid *l* or *d*-Tartaric "	D- or L- Threaric Acid
		Trihydroxyglutaric Acids	**Pentaric Acids**
D- or L-Ribose	Ribitol or Adonitol	*ribo* Trihydroxyglu taric Acid	Ribaric Acid
D or L Xylose	Xylitol	*xylo*-Trihydroxyglu taric Acid	Xylaric "
D or L Arabinose	D or L-Arabitol	D or L *arabo*-Trihy droxyglutaric Acid	D or L Arabaric Acid
D- or L-Lyxose	D or L " or D or L-Lyxitol	D or L-*arabo* Trihy droxyglutaric Acid	D- or L- Arabaric Acid
		Saccharic Acids	**Hexaric Acids**
D or L Galactose	Dulcitol or Galactitol	Mucic Acid	Galactaric Acid
D- or L-Allose	Allitol (Alloduler tol)	Allomucic Acid	Allaric "
D or L Mannose	D or L Mannitol	D or L *manno* Sac charic Acid	D or L- Mannaric Acid
D or L-Idose	D or L Iditol	D or L *ido*-Saccharic Acid	D- or L- Idaric Acid
D or L-Talose	D or L Talitol	D or L Talomucic Acid	D or L Talaric Acid
D- or L Altrose	D or L-Talitol or D or L Altritol	D or L Talomucic Acid	D or L Talaric Acid
D Glucose	Sorbitol (D Sorbitol)	(D *gluco*-)Saccharic Acid	D Glucaric Acid
L-Gulose	Sorbitol (D Sorbitol or L-Gulitol	"	" "
L-Glucose	D Gulitol (L Sorbitol)	(L *gluco*)Saccharic Acid	L " "
D-Gulose	D Gulitol (L-Sorbitol)	(L *gluco*)Saccharic Acid	L " "

been used for rhamnose, fucose and isorhamnose, but they are not used as frequently as the established trivial names, or the systematic 6-deoxy-hexose designations.

The dibasic acids are quite irregular. "Tartaric acid" is generic for the two active forms, the *meso*, and the D,L or *racemic* acid. The five-carbon acids are still called trihydroxyglutaric acids. In the six-carbon acids, known

as saccharic acids, three are termed *ido-*, *manno-* and *gluco-*saccharic acids, and the remaining three as mucic, allomucic and talomucic acids. The seven and higher-carbon series follow the higher sugar nomenclature.

Peirce[2] has suggested "aric acids", as generic for all dibasic sugar acids. "L-Threaric" is an unambiguous systematic equivalent for "L(dextro)-tartaric". "Glucaric" and "mannaric" have obvious advantages over "*gluco-*saccharic" and "*manno-*saccharic" but as yet this type of nomenclature has not received general acceptance.

C. D- AND L-Series. Carbohydrates are assigned to the D- or L-series, according to the configuration of the highest numbered chain asymmetric carbon atom in the chain, i.e., the one farthest from the principal function · aldehydo, keto, or carboxyl, etc. D- and L-Glyceraldehyde are accepted as the reference standard for carbohydrates, following Rosanoff's proposal.

$$
\begin{array}{cc}
\text{CHO} & \text{CHO} \\
| & | \\
\text{HCOH} & \text{HOCH} \\
| & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
\text{D-} & \text{L-}
\end{array}
$$
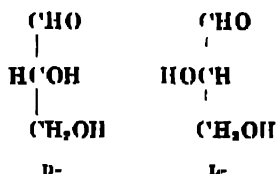
The higher sugars, and compounds derived therefrom, are all considered as being descendents of these two enantiomorphs, so that the D- or L-family assignment goes by the configuration on carbon 5 in hexoses, carbon 4 in pentoses, etc. Meso compounds, optically inactive by virtue of internal symmetry, have no family assignment. Like-ended compounds such as sorbitol having the same configurations for the terminal asymmetric carbon atoms may be considered as belonging to both series according to the carbon chosen as the point of reference.

D. Alpha-Beta Designations. The two anomeric forms of cyclic sugars or derivatives thereof are called α- and β-isomers. These isomers differ only in the configuration of the reducing (hemiacetal) group.

In the generally accepted Hudson system, which applies only to cyclic forms, the more *dextrorotatory* anomer of a D compound is called α,D; the other being β,D. The more *levorotatory* isomer of an L compound is called α,L, and the other β,L. The α-forms of enantiomorphs are therefore mirror images. Water or chloroform is generally used as solvent, and $[\alpha]_D$ is understood.

Many correlations have been established between α, β assignment and configuration, but, as applied generally to aldoses and ketoses, and their various functional derivatives, the assignment is still essentially on the basis of rotational behavior and family, as explained in the next chapter, the assignment has a configurational significance.

[2] G. Peirce, *J. Biol. Chem.*, **23**, 327 (1915).

The configuration of carbon 1 of α-D-glucose is accepted as being the same as that of methyl α-D-glucoside, but the anomeric configurations of most other free sugars have not been established unequivocally. There is no accepted primary reference compound for anomeric configuration, corresponding to D- and L-glyceraldehyde for family assignment.

Alpha and beta were also used by E. Fischer for naming the two 2-epimeric sugars obtained by the cyanohydrin synthesis from the hexoses or higher sugars. The first of a pair to be isolated was called α. Configurations were established later.

D-α-Glucoheptose

D Glucose

D-β-Glucoheptose
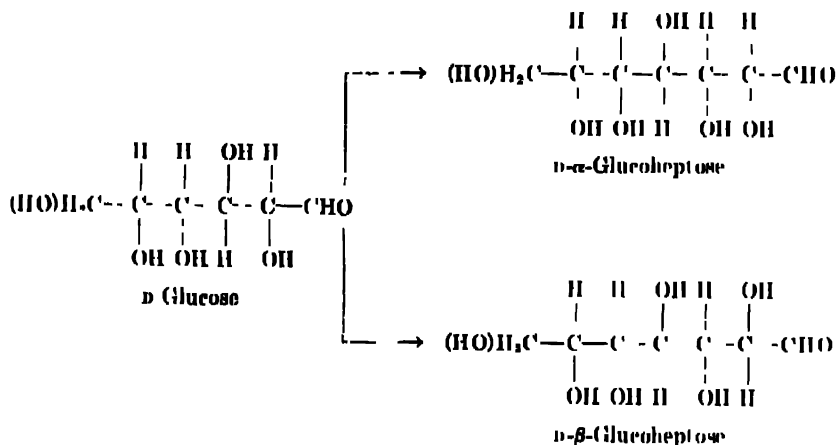
Here the α and β are part of the configurational name. The α and β anomers of each can exist. The use of α and β for this purpose is being superseded by other systems of nomenclature for the higher sugars (see Chapter II).

Other isomeric carbohydrate derivatives originally of unknown structure have been distinguished in the same way. Thus, there are the α- and β-di-isopropylidene-fructoses, the α- and β-dextrins and the α- and β-amyloses.

The α- and β-glycosidases are enzymes which can hydrolyze α- and β-glycosides, respectively. The α- and β-amylases are two types of diastatic enzymes

In view of the special significance of α and β in carbohydrates, it is preferable to use carbon numbers, rather than the α, β, γ system, for locating substituents Thus glucosamine is 2-desoxy-2-aminoglucose; 2-desoxyribose is used rather than α-desoxyribose and 6-desoxyglucose rather than ω-desoxy.

The symbols γ and δ are now used only for denoting 5- and 6-membered lactones of the aldonic acids. Ring size for both sugars and lactones is also indicated by the numbers of the carbon atoms concerned, e.g., 1,4; 3,6-D-saccharodilactone; 1,5;1,2-D-glucosan (= 1,2-anhydroglucopyranose). The term "γ" has been used to designate furanose (1,4) modifications and orthoester derivatives of sugars.

STRUCTURE AND STEREOCHEMISTRY OF THE MONOSACCHARIDES

1. Structures of Glucose and Fructose

The structure of glucose is established by the following evidence.[1] Dumas (1843) determined the empirical formula of the sugar to be CH_2O (when water is taken as H_2O and not as HO as it appears in the early work). Berthelot established the presence of a number of hydroxyl groups by the preparation of an acetate (indicated by him to be a hexaacetate) and formulated glucose as a hexahydric alcohol; however, as a result of additional studies (1862), glucose was formulated as an aldehyde-alcohol with five carbon atoms. The six carbon nature and the various known properties of glucose were expressed by Fittig and by Baeyer (1868 to 1870) in the formula:

$$(HO)H_2C \cdot CH(OH) - CH(OH) - CH(OH) - CH(OH) \cdot CHO \quad (Fittig, Baeyer)$$

The Baeyer-Fittig formula is confirmed by molecular weight determinations (B. Tollens and Mayer 1888), by the formation of pentaacetates and other esters and by the exhibition of many aldehyde-type reactions. Thus, the reduction of the sugar produces a hexahydric alcohol (sorbitol), and oxidation with bromine or nitric acid produces a monobasic acid (gluconic acid). These reactions would be anticipated from the presence of an aldehyde group. By reduction (with hydrogen iodide) of the alcohol or acid obtained from glucose, sec-hexyl iodide or n-hexylic acid is obtained. The formation of the sec-hexyl iodide proves that the sugar has a straight chain. These and many other reactions support the Baeyer-Fittig formulation of glucose. However, as will be shown below, the formula does not show the stereochemical relationships of the various groups, and many reactions and properties of the sugar are not fully expressed.

The presence in honey of a sirupy sugar different from glucose was recognized by many early workers, but the crystalline material was prepared first by Jungfleisch and Lefranc in 1881. The name of levulose seems to have been applied first by Berthelot (1860), whereas Emil Fischer (1890) suggested the name of fructose for this sugar.

Fructose must be constituted similarly to glucose, for it is reduced to hexahydric alcohols (mannitol and sorbitol). The mannitol has a straight chain structure as is shown by its conversion to sec-hexyl iodide by the

---

[1] For references see. "Beilsteins Handbuch der organischen Chemie," Vol. 31, p. 83; Julius Springer, Berlin (1938).

action of hydrogen iodide. Oxidation of the sugar with nitric acid yields *meso*-tartaric acid (COOH CHOH CHOH COOH), glycolic acid (CH$_2$OH COOH) and oxalic acid and must take place by cleavage of the carbon chain. The formation of tartaric acid and glycolic acid would be expected if a ketone group is present at carbon 2. The existence of a ketone group is shown by the formation of a branched-chain acid when fructose is treated with HCN. The nature of the seven-carbon acid formed by the addition of HCN was shown by Kiliani who reduced it to 2-methylhexanoic acid. The original formula for fructose as proposed by Krusemann (1876) and Kiliani (1881) is given below with its transformation to 2-methyl-hexanoic acid.

CH$_2$OH		CH$_2$OH		CH$_3$
CO		HOC—COOH		CH—COOH
CHOH	HCN	CHOH	HI P	CH$_2$
CHOH		CHOH		CH$_2$
CHOH		CHOH		CH
CH$_2$OH		CH$_2$OH		CH
Fructose (Krusemann and Kiliani)				2-Methylhexanoic acid
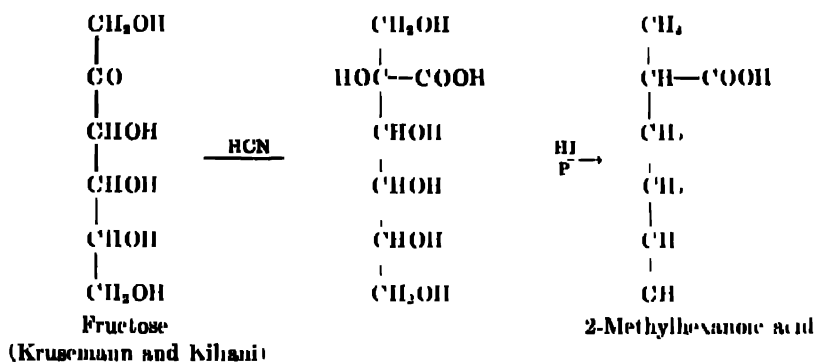
2. Stereochemistry

A. General Principles. The sugars with the formula C$_6$H$_{12}$O$_6$ known in 1886 were glucose, fructose, galactose, and sorbose. Of the known hexoses, two types of structures were present. These types were the glucose galactose type with aldehyde structures and the fructose-sorbose type with ketone structures.

The occurrence of structurally identical sugars such as glucose and galactose presented a challenge to the chemists of the later nineteenth century to provide an explanation for the existence of isomers of a type other than structural isomers. The basis for this explanation was developed almost simultaneously by Le Bel and van't Hoff and published in 1874. According to these workers, isomers of a type other than structural isomers should exist for compounds which contain asymmetric carbon atoms. This type of isomerism is illustrated below for glyceraldehyde (CH$_2$OH –CHOH —CHO). Each of the two isomers is represented by a tetrahedral formula and by a conventional formula.

The conventional formulas are derived from the tetrahedral formulas by the use of the convention established by Fischer.[2] The tetrahedrons are represented as being

---

[2] E. Fischer, *Ber.*, **24**, 1836, 2683 (1891).

held so that the dotted lower edge is in the plane of the paper; the H and OH corners are above the plane of the paper with the aldehyde group at the top. The conventional formula represents the projection of the model on the plane of the paper

CHO
|
HCOH
|
CH₂OH

CHO
H—<>—OH
CH₂OH

CHO
|
HOCH
|
CH₂OH

CHO
HO—<>—H
CH₂OH
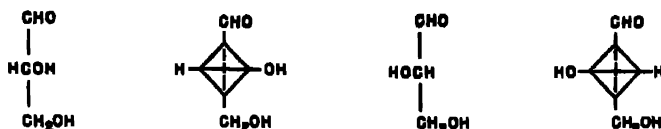
The two tetrahedrons differ only in the configuration of the groups in space, and the substances are called stereoisomers. Careful examination of the above figure, or better of models, will show that no matter how the tetrahedrons are turned in space they cannot be made to coincide. However, it should be noted that the two tetrahedrons are related in a fashion like that of an object and its mirror image When two of the groups attached to the same carbon are identical, isomerism of this type is not possible. The presence of asymmetric carbon atoms in organic compounds was suggested by Le Bel and van't Hoff as the cause of the optical activity of the compounds. Compounds which contain such atoms cause a rotation of the plane of polarization of plane-polarized light when the light is passed through their solutions.

For each of the trioses shown above, there are two related tetroses. The tetroses have two asymmetric carbon atoms; the formulas of the four possible isomers are given below in both the tetrahedral and the ordinary formulas.

The Fischer Configurational Formulas for the Four Tetroses

CHO
HO—<>—H
H—<>—OH
CH₂OH

CHO
H—<>—OH
H—<>—OH
CH₂OH

CHO
HO—<>—H
HO—<>—H
CH₂OH

CHO
H—<>—OH
HO—<>—H
CH₂OH

CHO
|
HOCH
|
HCOH
|
CH₂OH

CHO
|
HCOH
|
HCOH
|
CH₂OH

CHO
|
HOCH
|
HOCH
|
CH₂OH

CHO
|
HCOH
|
HOCH
|
CH₂OH

(I)   (II)   (III)   (IV)
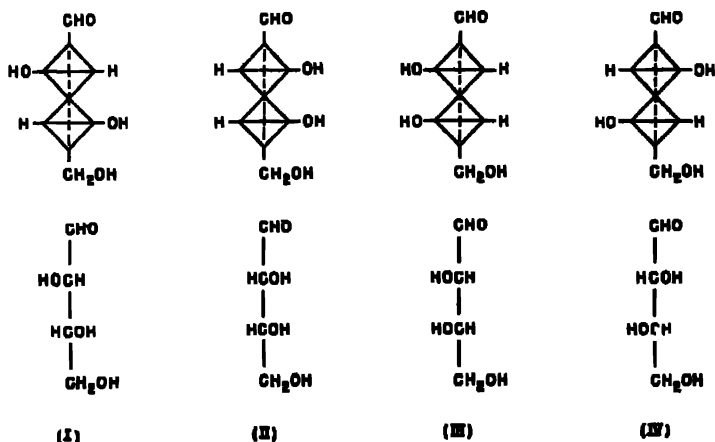
The isomeric tetroses differ in their spacial relationships and cannot be brought into coincidence by rotation of the models in space even though free rotation about the bond between the tetrahedra is possible. The

formulas I and IV are a pair of mirror images; II and III represent another
such pair. For the four-carbon sugars, there are two pairs of mirror images
(enantiomorphs) and four stereoisomers. In the sugar series, substances
which differ only in the configuration of the carbon atom immediately
adjacent to that carrying the carbonyl or carboxyl group are known as
epimers. In the above formulas, I and II represent a pair of epimers and
III and IV another pair. It may be well to extend the definition of epimers
to mean any pair of stereoisomers that differ solely in the configuration of a
single asymmetric carbon atom. By this definition compounds V and VI
would be 2-epimers and compounds V and VII would be 3-epimers.

$$
\begin{array}{ccc}
\text{HCO} & \text{HCO} & \text{HCO} \\
| & | & | \\
\text{HCOH} & \text{HOCH} & \text{HCOH} \\
| & | & | \\
\text{HOCH} & \text{HOCH} & \text{HCOH} \\
| & | & | \\
\text{HCOH} & \text{HCOH} & \text{HCOH} \\
| & | & | \\
\text{H}_2\text{COH} & \text{H}_2\text{COH} & \text{H}_2\text{COH} \\
\text{(D-Xylose)} & \text{(D-Lyxose)} & \text{(D-Ribose)} \\
\text{(V)} & \text{(VI)} & \text{(VII)}
\end{array}
$$
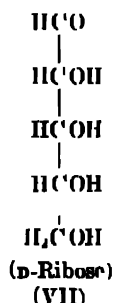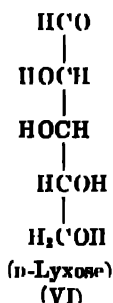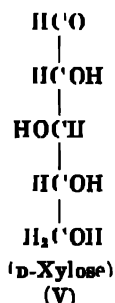
In general, the number of stereoisomers for a structure which involves n
asymmetric carbon atoms is given by 2^n. However, when the terminal
groups in the molecule are identical, the number of isomers is given by:
2^{1} $(2^{\frac{1}{2}}+1)$ when n is an even number, and by 2^{n-1} when n is an odd number
Thus, for the tartaric acids (COOH– CHOH CHOH COOH), three
isomers are possible, for the pentaric (hydroxyglutaric) acids (COOH
CHOH CHOH– CHOH– COOH), four isomers are possible. Fewer
isomers can exist when the end groups are identical because of the sym-
metries which develop. Thus in the compounds which have an odd number
of asymmetric carbon atoms, the central carbon has two attached groups
which may have the same structure. If two groups are identical, the number
of asymmetric centers is really $n - 1$. This relationship may be seen from
the formula given below for the pentaric (trihydroxyglutaric) acids.

$$
\begin{array}{ccccc}
\text{COOH} & & & & \\
| & & \text{COOH} & \text{COOH} & \text{COOH} & \text{COOH} \\
\text{CHOH} \quad \text{R} & & | & | & | & | \\
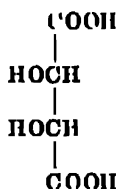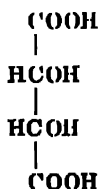| \qquad | & & \text{HCOH} & \text{HOCH} & \text{HCOH} & \text{HOCH} \\
\text{CHOH} = \text{CHOH} & & | & | & | & | \\
| \qquad | & & \text{HOCH} & \text{HCOH} & \text{HCOH} & \text{HOCH} \\
\text{CHOH} \quad \text{R} & & | & | & | & | \\
| & & \text{COOH} & \text{COOH} & \text{COOH} & \text{COOH} \\
\text{COOH} & & \text{(I)} & \text{(II)} & \text{(III)}
\end{array}
$$
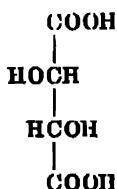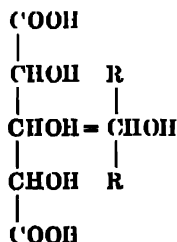
Trihydroxyglutaric acids   The isomeric tartaric acids

For the tartaric acids, which have an even number of carbon atoms, the number of isomers is reduced to three because of the symmetry of the molecule. The two formulas represented by III are identical. This identity may be shown by moving either of formulas III through 180°, keeping it in the plane of the paper. It then becomes identical with the other formula. When formula I is rotated in the plane of paper through 180°, it does not become identical with either II or III. A better test is provided by the construction of the space models; if this is done, it will be found possible to construct only three stereoisomers. Note, however, that any mono-substitution of III destroys the *meso* symmetry, giving rise to enantio-morphs.

In general, compounds which contain asymmetric carbon atoms rotate the plane of polarization of plane-polarized light. For this reason they are said to be optically active. When the molecular symmetry is such that the optical activity of one portion of the molecule is cancelled by that of the second portion of the molecule, the compounds are said to be internally compensated and are called *meso* compounds. The tartaric acid with the formula III is such a compound and is known as the *meso* tartaric acid. The tartaric acids identified as I and II have been known as *d*-tartaric acid and *l*-tartaric acid because of the sign of their optical rotations (*dextro* and *levo*, respectively). (The nomenclature of these acids is discussed later in this chapter.) The compounds I and II are mirror images (also called enantiomorphs) and have identical properties such as melting points, solubilities, etc. They differ only in the sign of their optical rotation and in their behavior towards asymmetric agents, *i.e.*, other optically active substances. Mixtures of equal amounts of the acids I and II are optically inactive and are termed racemic or D,L mixtures. The optical inactivity of such mixtures arises from "external compensation" as distinguished from the "internal compensation" of the *meso* form. Racemic mixtures are important because they are always produced in the chemical synthesis of potentially active substances from inactive materials unless asymmetric substances have been used in the synthesis. The widespread occurrence of optically active substances in natural products usually is to be ascribed to the asymmetry of the natural catalysts, usually enzymes.

On the basis of the above considerations, which are consequences of the Le Bel-van't Hoff theory, the number of isomers of each of the sugars having seven or less carbon atoms and of the corresponding dibasic acids and alcohols is given in Table I.

B. Establishment of the Configuration of Glucose and Some Other Sugars. The existence of structurally isomeric sugars was a corollary of the Le Bel-van't Hoff theory. After publication of the theory in the latter part of the nineteenth century, it was soon realized that sugars such as glucose and galactose are stereoisomers. In a series of brilliant researches, Emil

Fischer applied the Le Bel-van't Hoff theory to the sugar series and established the configurations of the individual sugars

Fischer's proof was published in two papers which appeared in 1891.[3] His proof was expressed in the terminology and conventions of the time. Since the expression of the proof in his original fashion would require a detailed explanation of the older concepts of stereochemistry, it seems better in the present discussion to use the data available to him at the time and to introduce the proof in terms of modern concepts and conventions The present discussion follows the proof of configuration as outlined[4] by C S. Hudson and in part quotes him

<div align="center">

TABLE I

Number of Stereoisomers of the Aldehyde Sugars and Aldonic Acids Containing 2 to 7 Carbons and of the Corresponding Alcohols and Dibasic Acids

(See also Table I of Chapter I)

</div>

		Number of possible forms or isomers	
Parent Sugar	No of asymmetric carbons (n)	Sugars (& Aldonic Acids) CHO (COOH) (CHOH)n CH₂OH	Alcohols (& Dibasic Acids)* CH₂OH (COOH) (CHOH)n CH₂OH (COOH)
Dioses	0	1	1
Trioses	1	2	1
Tetroses	2	4	3
Pentoses	3	8	4
Hexoses	4	16	10
Heptoses	5	32	16

* When n is an odd number one carbon is not asymmetric

The following facts were available to Fischer at the time of his establishment of the configuration of glucose

(1) Three sugars with the formula $C_6H_{12}O_6$ (D-glucose, D-mannose and D-fructose) react with an excess of phenylhydrazine to give the same product, glucose phenylosazone The reactions are illustrated in the accompanying formulas

Carbon No.

```
1     CHO                          HC=N  N  C₆H₅
      |                                 |
2     CHOH          C₆H₅NH_NH₂       C=N- NHC₆H₅
      |              ────────→          |
3-5   (CHOH)₃                       (CHOH)₃
      |                                 |
6     CH₂OH                         CH₂OH
      Glucose and
      Mannose
```
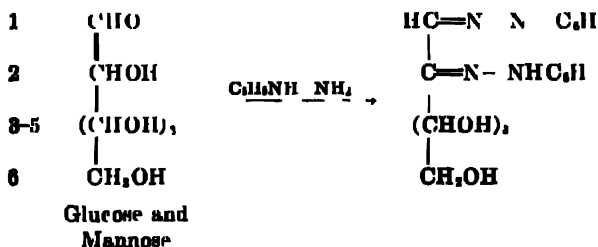
[3] E. Fischer, *Ber*, **24**, 1836, 2683 (1891).
[4] C. S. Hudson, *J. Chem Education*, **18**, 353 (1941).

$$
\begin{array}{ll}
HC=\!N-NHC_6H_5 & CH_2OH \\
| & | \\
C=\!N-NHC_6H_5 & CO \\
| & | \\
(CHOH)_3 & (CHOH)_3 \\
| & | \\
CH_2OH & CH_2OH
\end{array}
$$

$\xleftarrow{\quad C_6H_5NH-NH_2 \quad}$

Glucose phenylosazone   Fructose
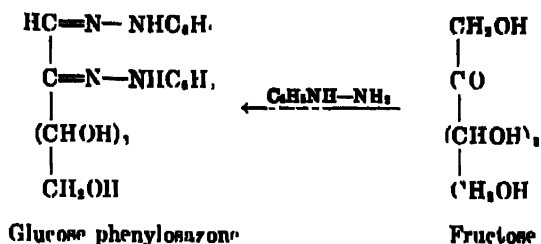
The above reactions prove that mannose and glucose are 2-epimers, *i.e.*, they differ only in the configuration of carbon atom 2; also, fructose, glucose and mannose must have the same configurations for carbon atoms 3, 4 and 5.

(2) Glucose and mannose are oxidized by nitric acid to dibasic acids which are different and which are both optically active.

$$
\begin{array}{lll}
CHO & & COOH \\
| & \xrightarrow{HNO_3} & | \\
(CHOH)_4 & & (CHOH)_4 \\
| & & | \\
CH_2OH & & COOH
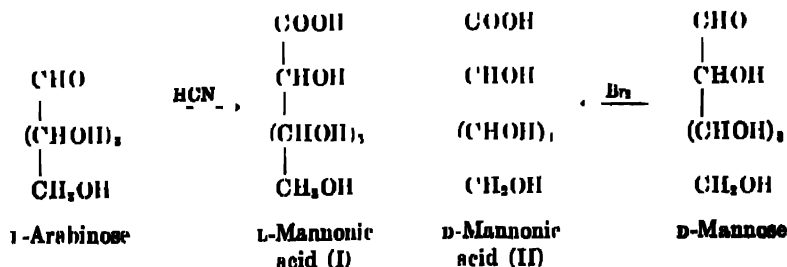\end{array}
\qquad
\begin{array}{lll}
CHO & & COOH \\
| & \xrightarrow{HNO_3} & | \\
(CHOH)_4 & & (CHOH)_4 \\
| & & | \\
CH_2OH & & COOH
\end{array}
$$

Glucose   gluco-Saccharic acid   Mannose   manno-Saccharic acid
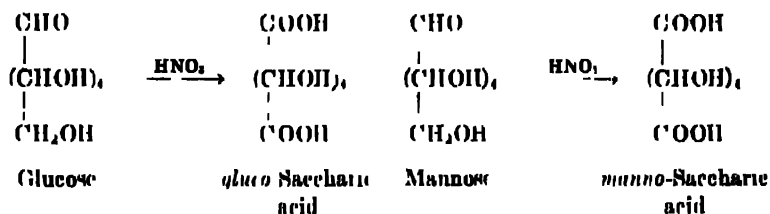
The optical activity of the products proves that the configuration of the asymmetric atoms (carbon atoms 2 to 5) cannot be of the type which produces internal compensation.

(3) L-Arabinose, which had been isolated from beet pulp by Scheibler in 1868 and shown to be an aldopentose by Kiliani in 1887, reacts with HCN with the production of a nitrile which hydrolyzes to a six-carbon monobasic acid (I). This acid was shown by Fischer to be the mirror image of the acid (II) produced by the mild oxidation of mannose.

$$
\begin{array}{llll}
CHO & \xrightarrow{HCN} & COOH & COOH & CHO \\
| & & | & | & | \\
& & CHOH & CHOH & CHOH \\
(CHOH)_3 & & | & | & | \\
| & & (CHOH)_3 & (CHOH)_3 & (CHOH)_3 \\
CH_2OH & & CH_2OH & CH_2OH & CH_2OH
\end{array}
$$

l-Arabinose   L-Mannonic acid (I)   D-Mannonic acid (II)   D-Mannose

In the synthesis of L-mannonic acid (I), a second acid also is formed which is enantiomorphous with that obtained by the oxidation of glucose.

The dibasic acid obtained by the nitric acid oxidation of the arabinose also is optically active.

(4) Saccharic acid not only can be obtained by the oxidation of D-glucose as indicated above, but it is also obtained by the oxidation of another hexose, L-gulose.

$$
\begin{array}{ccc}
\text{HCO} & \text{COOH} & \text{COOH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{HOCH} & \text{HOCH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{H}_2\text{COH} & \text{H}_2\text{COH} & \text{COOH} \\
\text{D-Glucose} & \text{D Gluconic acid} & \text{D-}gluco\text{-Saccharic acid}
\end{array}
$$

$$
\text{D-Glucose} \xrightarrow{\text{Br}_2} \text{D Gluconic acid} \xrightarrow{\text{HNO}_3} \text{D-}gluco\text{-Saccharic acid}
$$

$$
\begin{array}{cccc}
\text{HCO} & \text{COOH} & \text{COOH} & \text{COOH} \\
\text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HCOH} \\
\text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & = & \text{HCOH} \\
\text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HCOH} \\
\text{H}_2\text{COH} & \text{H}_2\text{COH} & \text{COOH} & \text{COOH} \\
\text{L-Gulose} & \text{L-Gulonic acid} & \text{D-}gluco\text{-Saccharic acid}
\end{array}
$$

$$
\text{L-Gulose} \xrightarrow{\text{Br}_2} \text{L-Gulonic acid} \xrightarrow{\text{HNO}_3} \text{D-}gluco\text{-Saccharic acid}
$$

(5) No generally acceptable method is available for the establishment of absolute configurations. The method used by Fischer, and described below, leads finally to a choice between either of a pair of configurations which have a mirror image relationship. Fischer's solution of this problem consisted in the arbitrary assignment to saccharic acid (derived from glucose) of one of two possible formulas. By this action a convention was established, which enabled him to make a choice between the enantiomorphous formulas for other substances, once their genetic relationships with saccharic acid or glucose had been established. Fischer's concept, although fundamentally correct, has been somewhat modified and made more precise (See discussion of D,L-usage later in this chapter.) In conformity with the modern concepts, the convention may be expressed by placing the hydroxyl

of carbon 5 of glucose on the right side of the carbon chain (see proof below). According to the convention, glucose then will be called D-glucose; because mannose and fructose have the same configurations for carbon 5, they also are known as D-mannose and D-fructose

The above facts were known at the time of Fischer and, in conjunction with the Le Bel-van't Hoff theory, enabled him to select the configuration of glucose from those for the eight configurations which are possible (when only one of each of the mirror images is considered). The following proof quoted from a paper by C. S. Hudson, may be said to be a modernized version of the Fischer proof.

"Write the formulas for a pentose (A) and the two hexoses (B and C) which it yields by the Fischer-Kiliani cyanohydrin synthesis as shown in the accompanying diagram (I), using Fischer's convention that the asymmetric carbon atoms (tetrahedra) have the lower edge in the plane of the paper and the corners which carry the H and OH groups lie above this plane. The arrangement of the H and OH groups is then decided through the following steps, in which the pentose is selected to be D-arabinose and in consequence the hexoses become D-glucose and D-mannose"


(I)
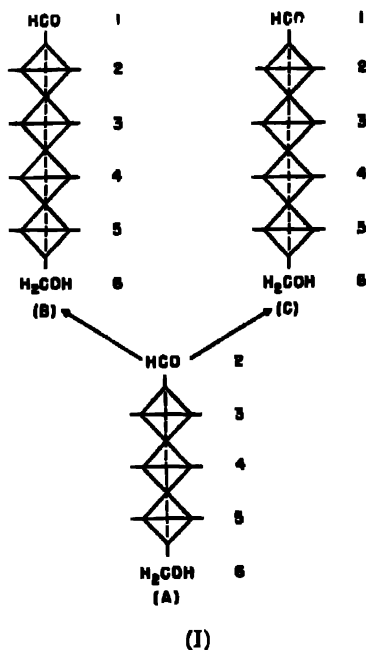
"Step 1   By convention for the D-configurational series OH is on the right of C-5 (see II).

"Step 2- (D) is optically active hence OH is on the left of C-3 (see II).

(II)
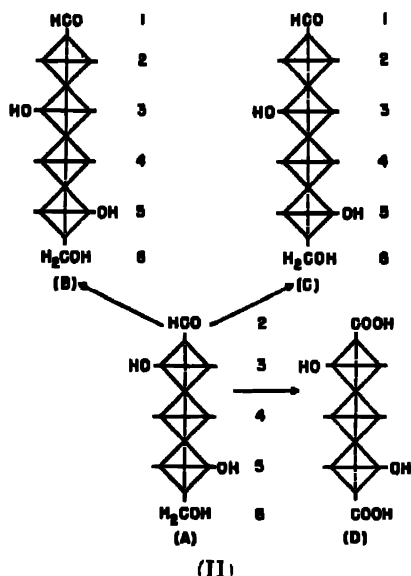
'Step 3   D-Glucose and D-mannose are epimeric, hence the OH's on C-2 are opposed. Either (B) or (C) may be selected as having OH on the right, without changing the final result, here the OH is placed to the right of C 2 in (B) and consequently to the left in (C) (see III)

(III)
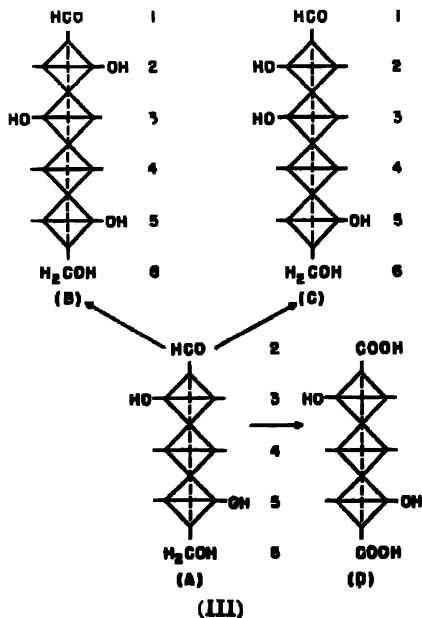
'Step 4—Since both saccharic and manno-saccharic acids (E and F) are optically active the configuration of neither of them can possess end-to-end symmetry; hence the OH on C-4 must be on the right (see IV). (If it were on the left, (E) would have end-to-end symmetry.)
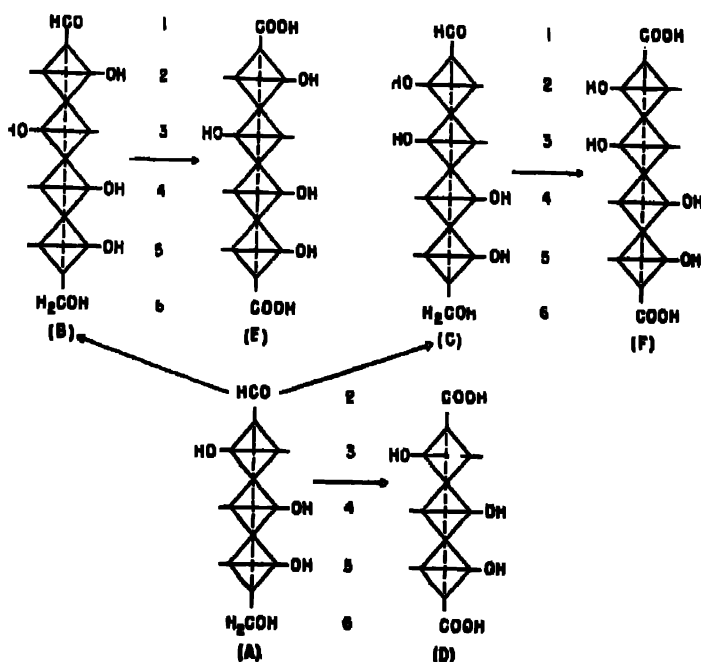
(IV)

At this stage the configuration of D-arabinose (A) and its dibasic acid (D) have become established. D-Glucose and D-mannose have been limited to the configurations (B) and (C), but the correlation within this limit remains to be established. This is done by:
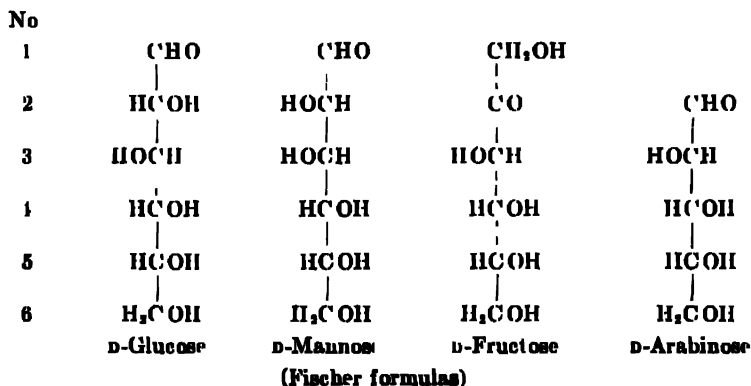
'Step 5   Saccharic acid is obtainable from the oxidation of each of two hexoses, namely glucose and gulose. (E) must therefore refer to D-saccharic acid because (F) cannot result from the oxidation of two hexoses   Hence (B) refers to D-glucose, (C) to D mannose, and (F) to D-manno-saccharic acid "

The proof is now complete and (V) the formulas become:

D-Glucose   D-gluco-Saccharic acid   D-Mannose   D-manno-Saccharic Acid

D-rabinose   D-arabo-Trihydroxy-glutaric acid

(V)
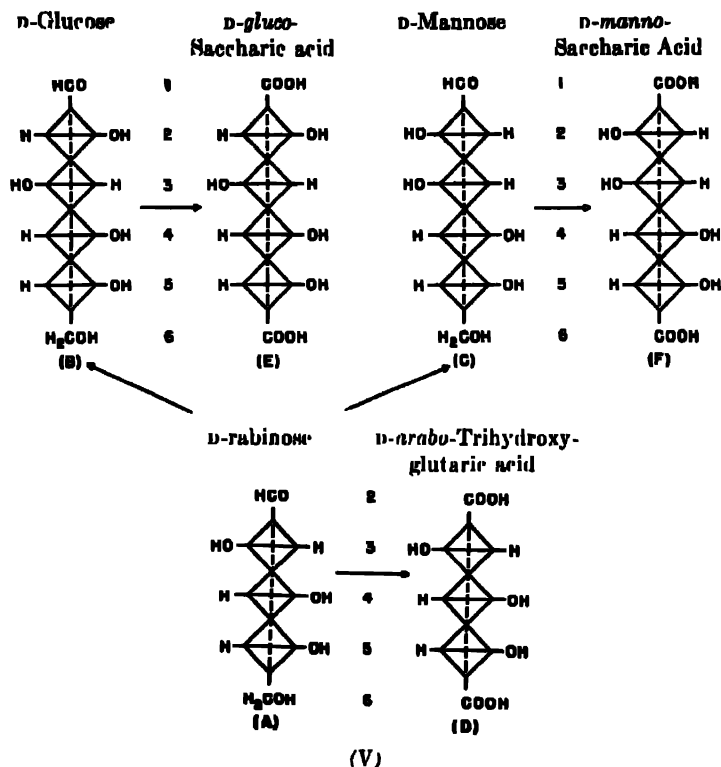
By means of the Fischer convention, the tetrahedral models for glucose, mannose and arabinose are equivalent to the planar formulas given below. The formula for fructose is derived from the fact that fructose yields the same osazone as glucose when treated with phenylhydrazine (see above); it and glucose must have identical configurations for carbon atoms 3, 4 and 5.

No				
1	CHO	CHO	CH₂OH	
2	HCOH	HOCH	CO	CHO
3	HOCH	HOCH	HOCH	HOCH
4	HCOH	HCOH	HCOH	HCOH
5	HCOH	HCOH	HCOH	HCOH
6	H₂COH	H₂COH	H₂COH	H₂COH
	D-Glucose	D-Mannose	D-Fructose	D-Arabinose

(Fischer formulas)

C. D and L-Nomenclature. In some types of optically active compounds, it has been customary to distinguish between the enantiomorphous modifications by indicating the sign of their optical rotation as "*d*" (dextrorotatory) or "*l*" (levorotatory). Thus, *d*-tartaric acid (the naturally occuring form) is the isomer which has a dextrorotation. This usage is not followed in carbohydrate chemistry except in exceptional instances. Fischer established the convention of calling ordinary glucose *d*-glucose and employed the prefix *d*- in a configurational sense to mean that a *d*-substance is derivable from *d*-glucose whereas an *l*-substance is derivable from *l*-glucose. Hence, fructose was called *d*-fructose although it exhibits a levorotation.

The Fischer system, however, was modified by Rosanoff[6] in order that certain ambiguities would be avoided. Thus, the following transformations have been carried out.

D-Glucose	D-Glucuronic acid	D-Xylose
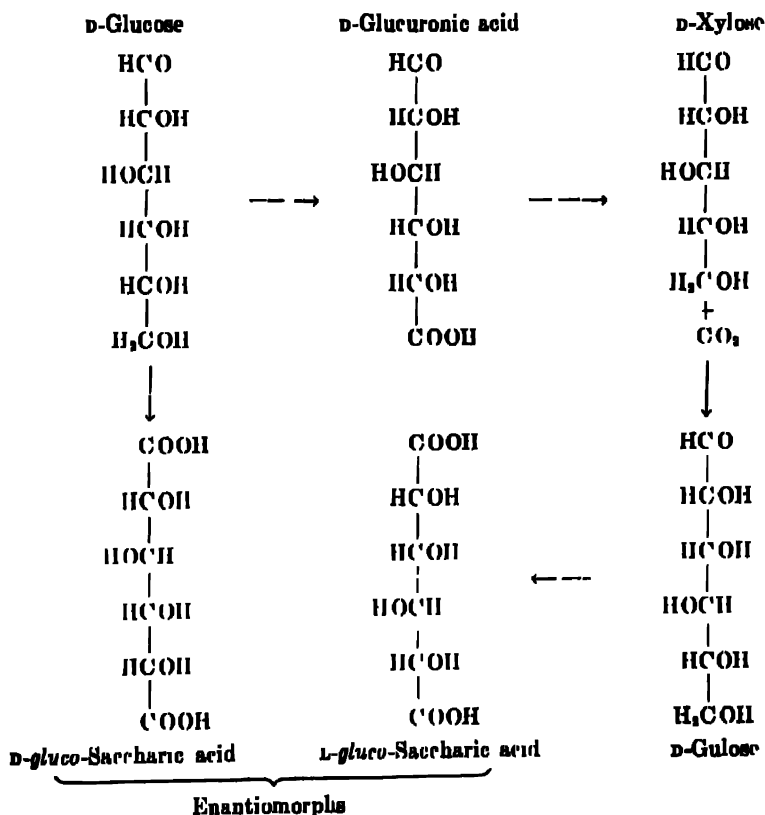
Either of the enantiomorphous forms of saccharic acid may be produced from ordinary glucose as shown above. Since the transformation of the D-

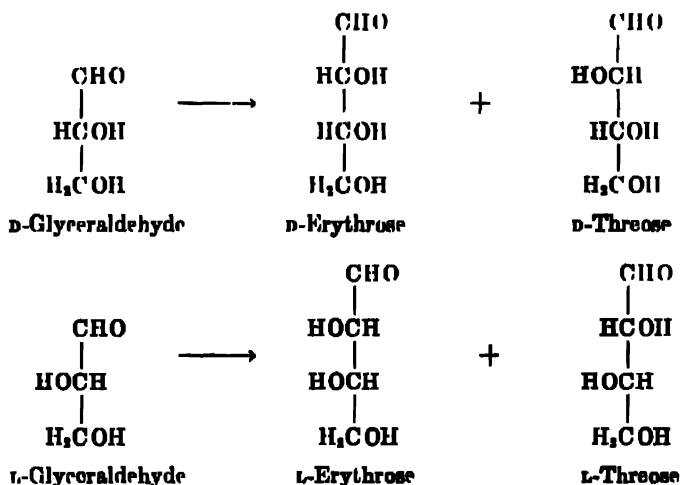6 M. A. Rosanoff, *J. Am. Chem. Soc.*, **28**, 114 (1906).

xylose (natural form) to a saccharic acid which is the mirror image of that obtained by the direct oxidation of the glucose was observed first, the natural xylose originally was called *l*-xylose by Fischer; if the conversion of glucose to xylose through glucuronic acid had been observed first, the natural sugar probably would have been termed *d*-xylose.

The system proposed by Rosanoff placed the use of the symbols *d* and *l* (or now D and L) on a logical genetic basis. His system is universally accepted by carbohydrate chemists. It starts with the definition that the glycerose which has the formula (I) shall be called D-glyceraldehyde and that with the formula (II) shall be called L-glyceraldehyde.

$$
\begin{array}{cc}
\text{CHO} & \text{CHO} \\
| & | \\
\text{HCOH} & \text{HOCH} \\
| & | \\
\text{H}_2\text{COH} & \text{H}_2\text{COH} \\
\text{D Glyceraldehyde} & \text{L Glyceraldehyde} \\
\text{(I)} & \text{(II)}
\end{array}
$$
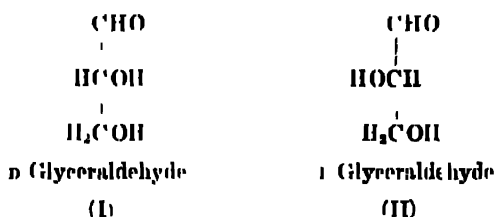
In the above names the capital letters D and L (small Roman capitals) are used rather than the small letters *d* and *l* This change seems to be gaining favor at least in American publications One reason for the change is to avoid confusion with *d* and *l* as used, particularly outside the field of carbohydrate chemistry, to indicate the sign of rotation rather than the configuration It is urged that the capital letters be retained only to indicate configurations and not the sign of rotation
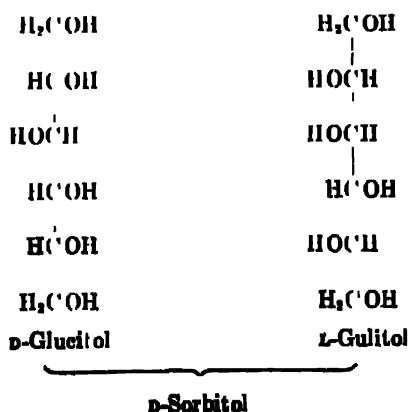
According to Rosanoff, all of the higher sugars which conceivably might be derived from D-glyceraldehyde by successive application of the cyanohydrin synthesis shall be called D-sugars. Similarly, all of those obtained in this manner from L-glyceraldehyde shall be called L-sugars.

$$
\begin{array}{ccccc}
 & & \text{CHO} & & \text{CHO} \\
 & & | & & | \\
\text{CHO} & & \text{HCOH} & & \text{HOCH} \\
| & & | & + & | \\
\text{HCOH} & \longrightarrow & \text{HCOH} & & \text{HCOH} \\
| & & | & & | \\
\text{H}_2\text{COH} & & \text{H}_2\text{COH} & & \text{H}_2\text{COH} \\
\text{D-Glyceraldehyde} & & \text{D-Erythrose} & & \text{D-Threose}
\end{array}
$$

$$
\begin{array}{ccccc}
 & & \text{CHO} & & \text{CHO} \\
 & & | & & | \\
\text{CHO} & & \text{HOCH} & & \text{HCOH} \\
| & & | & + & | \\
\text{HOCH} & \longrightarrow & \text{HOCH} & & \text{HOCH} \\
| & & | & & | \\
\text{H}_2\text{COH} & & \text{H}_2\text{COH} & & \text{H}_2\text{COH} \\
\text{L-Glyceraldehyde} & & \text{L-Erythrose} & & \text{L-Threose}
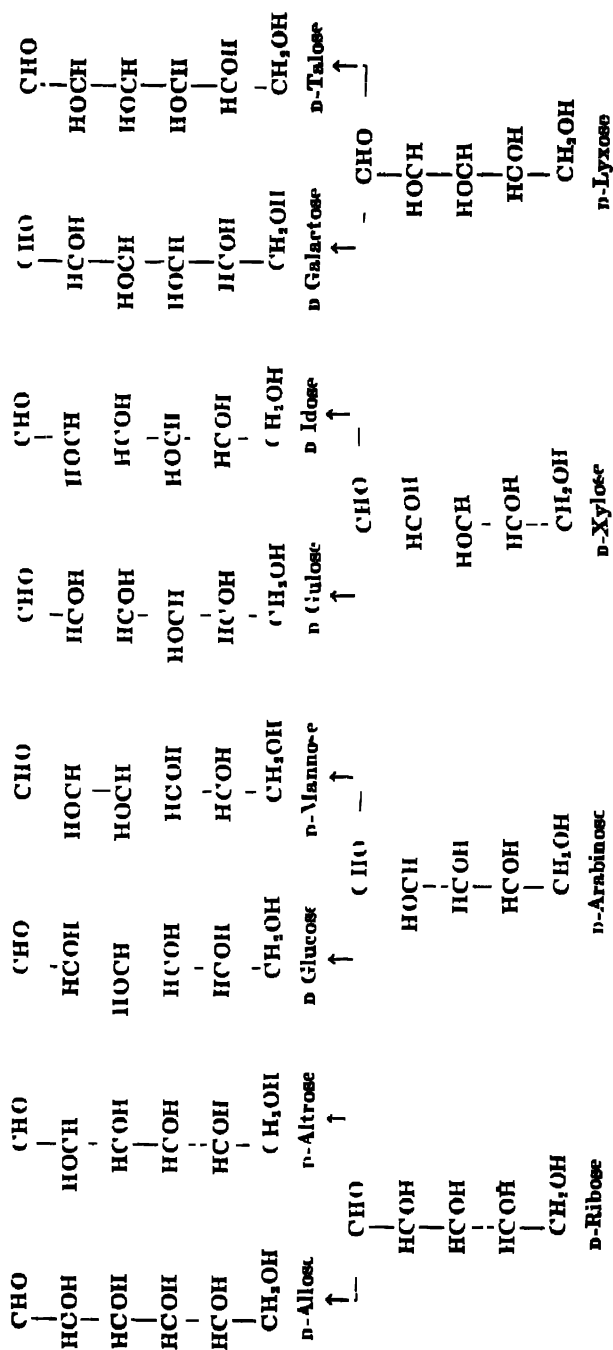\end{array}
$$

Since a new asymmetric carbon atom is produced in the addition of a carbon atom (through the cyanohydrin synthesis), two epimers are produced from each of the glyceroses. A continuation of this process with each of the four-carbon sugars conceivably would give four D-pentoses and four L-pentoses; application of the cyanohydrin synthesis to the pentoses produces in turn eight D- and eight L-hexoses. Although this entire process has not been carried out experimentally, interconversions have been carried out in number sufficient for the allocation of the configurations of all of the possible sugars through the hexose stage and for many of the higher sugars.

In general, substances may be defined as belonging to the D-family when the asymmetric carbon atom most remote from the reference group (*e.g.*, aldehyde, keto, carboxyl, etc.) has the same configuration as in D-glyceraldehyde; if this carbon has the same configuration as that in L-glyceraldehyde, the substance belongs to the L-family. When the compound is written in the Fischer manner with the reference group towards the top, the allocation to the D- or L series is made on the basis of the configuration of the bottom-most asymmetric carbon atom, usually the penultimate carbon; substances of the D-series have the hydroxyl group lying on the right and of the L-series on the left. When two possible reference groups are present in the same molecule, the choice of reference group is usually in the following order: CHO, COOH, CO (ketone); for example, in D-glucuronic acid, the reference group is the aldehyde group rather than the carboxyl group

This classification leads to ambiguous assignment in the case of certain optically active, like ended compounds wherein the end asymmetric carbons have the same configuration Such compounds must have a minimum chain length of six carbon atoms, and of those with six carbon atoms only the glucose (sorbitol) configuration leads to ambiguity. Thus, D-sorbitol might be called D-glucitol or L-gulitol
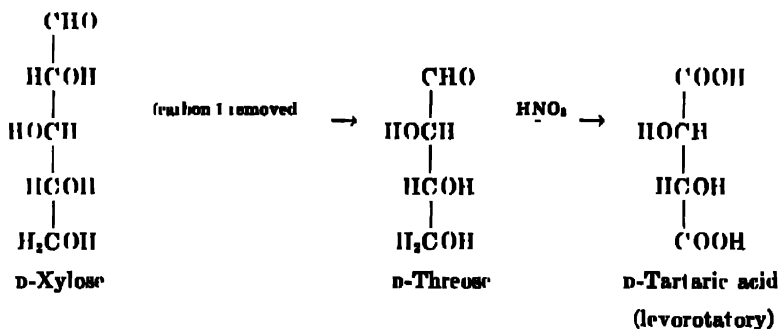
H₂COH	H₂COH
HCOH	HOCH
HOCH	HOCH
HCOH	HCOH
HCOH	HOCH
H₂COH	H₂COH
D-Glucitol	L-Gulitol

D-Sorbitol

```
CHO         CHO          CHO          CHO          CHO          CHO          CHO          CHO
|           |            |            |            |            |            |            |
HCOH        HOCH         HCOH         HOCH         HCOH         HOCH         HCOH         HOCH
|           |            |            |            |            |            |            |
HCOH        HCOH         HOCH         HOCH         HCOH         HCOH         HOCH         HOCH
|           |            |            |            |            |            |            |
HCOH        HCOH         HCOH         HCOH         HOCH         HOCH         HOCH         HOCH
|           |            |            |            |            |            |            |
HCOH        HCOH         HCOH         HCOH         HCOH         HCOH         HCOH         HCOH
|           |            |            |            |            |            |            |
CH2OH       CH2OH        CH2OH        CH2OH        CH2OH        CH2OH        CH2OH        CH2OH
D-Allose    D-Altrose    D-Glucose    D-Mannose    D-Gulose     D-Idose      D-Galactose  D-Talose

      CHO          CHO          CHO          CHO
      |            |            |            |
      HCOH         HOCH         HCOH         HOCH
      |            |            |            |
      HCOH         HCOH         HOCH         HOCH
      |            |            |            |
      HCOH         HCOH         HCOH         HCOH
      |            |            |            |
      CH2OH        CH2OH        CH2OH        CH2OH
      D-Ribose     D-Arabinose  D-Xylose     D-Lyxose
```

CHO
|
HCOH
HCOH
CH₂OH
D-Threose

CHO
|
HCOH
HCOH
CH₂OH
D-Erythrose

CHO
|
HCOH
CH₂OH
D-Glyceraldehyde

The D-Family of Aldoses Having 3 †, 6 Carbon Atoms

Since sorbitol is a trivial name (like sucrose or lactose) given to the naturally occurring compound before the configuration was known, it may be used properly without the D- specification as the name of the naturally occurring isomer.

Either end of the chain can be considered to be the principal function so that the configuration at either carbon 2 or carbon 5 would make the family assignment L or D. Compounds of this type, called *amphi* by Rosanoff, are more numerous in the higher carbon series. *gluco*-Saccharic acid is another important example. In practice, sorbitol and *gluco*-saccharic acid have inherited the D-assignment from the more important parent, which is also the one from which the compounds were first obtained.

In one of the accompanying diagrams, the configurations of the D-aldoses which have six or less carbons in the molecule are illustrated on the basis of a genetic relation with the D-glyceraldehyde, although, as mentioned, the complete derivation of the pentoses and hexoses from the glyceraldehydes has never been accomplished. The other diagram shows the configurations of the D-ketoses with six or less carbons in the molecule and their configurational relation to the parent D-erythrulose.

Because many optically active substances can be related to the tartaric acids, it is desirable to relate the configurations of the sugars to these acids. This correlation was accomplished first by Fischer,[6] but it will be illustrated by the conversions carried out by Hockett:[7]


```
     CHO
      |
     HCOH                                  CHO                        COOH
      |          carbon 1 removed           |           HNO₃           |
     HOCH       ──────────────→   →        HOCH      ─────────   →    HOCH
      |                                     |                          |
     HCOH                                  HCOH                       HCOH
      |                                     |                          |
     H₂COH                                 H₂COH                      COOH
    D-Xylose                             D-Threose                D-Tartaric acid
                                                                  (levorotatory)
```

The configuration of the levorotatory tartaric acid is established by this process. In conformity with the Rosanoff system, it should be known as D-tartaric acid, but it usually is described by its original name of *l-(levo)*-tartaric acid which was given because of its levorotation. The naturally occurring form is the dextrorotatory, L-tartaric acid, or earlier, *d*-tartaric acid. In order to avoid confusion, the configurational names D- and L-threaric acids might be used.

[6] E. Fischer, *Ber.*, *39*, 1377 (1896).
[7] R. C. Hockett, *J. Am. Chem. Soc.*, *57*, 2260 (1935)

D-Family of Ketoses


```
    CH₂OH            CH₂OH            CH₂OH            CH₂OH
      |                |                |                |
     C=O              C=O              C=O              C=O
      |                |                |                |
    HCOH             HOCH             HCOH             HOCH
      |                |                |                |
    HCOH             HCOH             HOCH             HOCH
      |                |                |                |
    HCOH             HCOH             HCOH             HCOH
      |                |                |                |
    CH₂OH            CH₂OH            CH₂OH            CH₂OH
  D Allulose       D-Fructose       D-Sorbose        D Tagatose
  D-Psicose        Levulose
      ↑                ↑                ↑                ↑

                   CH₂OH                             CH₂OH
                     |                                 |
                    C=O                               C=O
                     |                                 |
                   HCOH                              HOCH
                     |                                 |
                   HCOH                              HCOH
                     |                                 |
                   CH₂OH                             CH₂OH
                 D-Ribulose                        D-Xylulose
                 (Adonose)                         D-Lyxulose
                     ↑                                 ↑

                            CH₂OH
                              |
                             C=O
                              |
                            HCOH
                              |
                            CH₂OH
                        D-Erythrulose
                        D-Threulose

                              |

                            CH₂OH
                              |
                             C=O
                              |
                            CH₂OH
                        Dihydroxyacetone
```
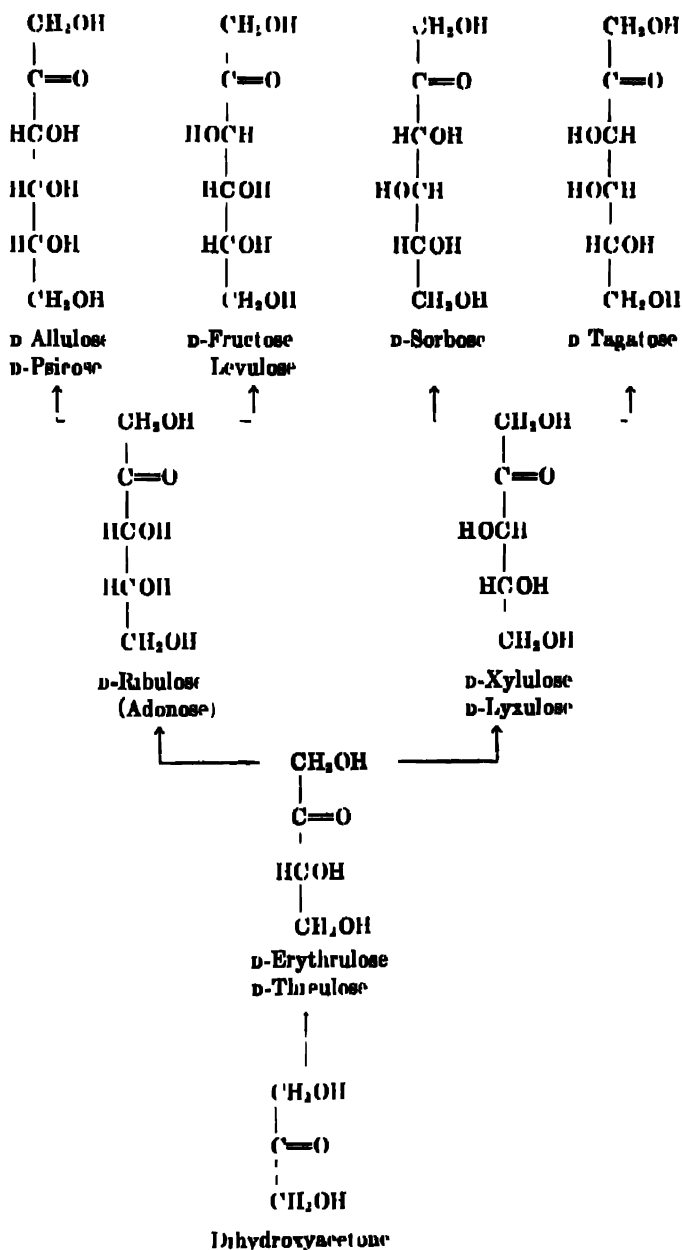
The related optically active erythritols were called *d* and *l* in the early literature although they have the L and D configurations, respectively

Likewise, Maquenne and Bertrand[a] called their erythrulose (from *meso*-erythritol) *d*-erythrulose.

Hockett gave *d*-erythrulose its configurational L-designation and likewise proposed D- and L-threitol for the optically active erythritols. The *meso* form is known as erythritol. The present authors use the configurational nomenclature for these compounds:

$$
\begin{array}{cccc}
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CHO} & \text{COOH} \\
| & | & | & | \\
\text{HCOH} & \text{CO} & \text{HCOH} & \text{HCOH} \\
| & | & | & | \\
\text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HOCH} \\
| & | & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{COOH}
\end{array}
$$

Carbohydrate name	ɪ Threitol	ɪ-Erythrulose	ʟ-Threose	ʟ-Tartaric acid (ʟ Threaric acid)
Technical or historical name	*d*-Erythritol	*d* Erythrulose	*l* Threose	*d* Tartaric acid

The biologically important sarcolactic acid is usually called *d*-lactic acid from its rotation, its carbohydrate name is L-lactic acid. The common *l*-malic acid is considered to have the configuration

$$
\begin{array}{c}
\text{H} \\
| \\
\text{HOOC} - \text{C} - \text{CH}_2 - \text{COOH} \\
| \\
\text{OH}
\end{array}
$$
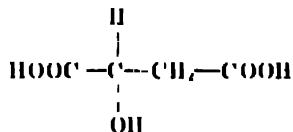
hence, its carbohydrate name would be 2-desoxy-D-tartaric acid.

3. Ring Structures of the Sugars

A. Necessity for Ring Structures. Soon after the formulation of glucose as a polyhydroxy aldehyde and of fructose as a polyhydroxy ketone, it became evident that the open-chain formulas would not account for all of the reactions of these sugars. Thus, the sugars give a negative test with the Schiff reagent (fuchsin and sulfurous acid) under the usual conditions of test although, under milder conditions, positive results are obtained.[b]

The aldehyde and ketone structures also do not account for the change of optical rotation which may be observed for the freshly prepared aqueous solutions of many sugars. This phenomenon, now called mutarotation, was observed by Dubrunfaut in 1846 for glucose solutions.

When the hydroxyl groups of glucose are esterified by treatment with acetic anhydride and a catalyst, two isomeric pentaacetates are formed.

[a] L. Maquenne and G. Bertrand, *Compt. rend.*, *132*, 1419 (1901).

[b] A. Villiers and M Fayolle, *Bull soc chim* . [3] *11*, 692 (1894); W C Tobie, *Ind. Eng Chem* , *14*, 405 (1942).

Similarly, two isomeric methyl glucosides are formed by treatment of glucose with methanol and hydrogen chloride. The existence of two gluco-sides[10] and two pentaacetates[11] cannot be predicted on the basis of the aldehyde formula, a conclusion first stated by Fischer in the case of the methyl glucosides, which he discovered.

The isolation of crystalline isomers of the sugars provided additional evidence for the inadequacy of the aldehyde formulas. As early as 1856, two crystalline modifications of lactose were prepared by Erdmann[12], the forms which are now designated α- and β-lactose; he discovered their mutarotations to the common equilibrium rotation. Tanret[13] in 1895 reported the isolation of three forms of glucose which he described as α-, β- and γ-glucose with the following rotations:

α-Glucose	"β-Glucose"	"γ-Glucose"
+106° – – – – →	+52.5' ← – – — – – —	+22.5'

When dissolved in water, the α-glucose mutarotated downward and the "γ-glucose" upwards to the same constant specific rotation of 52.5°. Tanret's "β-glucose" exhibited no mutarotation and later was considered to be a mixture of the two other forms in their equilibrium proportions. The name of β-glucose is now given to the form which he named as "γ-glucose." The common form is the α-isomer.

Even before the various isomers of glucose and its derivatives had been isolated, the absence of some typical aldehyde reactions for glucose had been explained by Colley (1870) and by Tollens (1883) as arising from a partial blocking of the aldehyde group by the formation of an inner hemi-acetal type of linkage. The formulas proposed by Colley and by Tollens are illustrated below.

Colley formula   Tollens formula
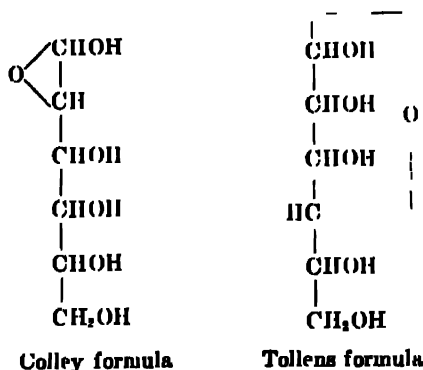
[10] E Fischer, Ber., 28, 1145 (1895).
[11] E Erwig and W Koenigs, Ber., 22, 1161, 2207 (1889)
[12] E. O. Erdmann, Ber, 13, 2180 (1880)
[13] C. Tanret, Compt. rend., 120, 1060 (1895).

The ring forms of the sugars represent intramolecular hemiacetal derivatives. Aldehydes react with alcohols with the formation of hemiacetals and acetals:

$$R-CHO \xrightarrow{R'OH} R-CH\begin{smallmatrix}OR'\\ \\OH\end{smallmatrix} \xrightarrow{R'OH} R-CH(OR')_2$$
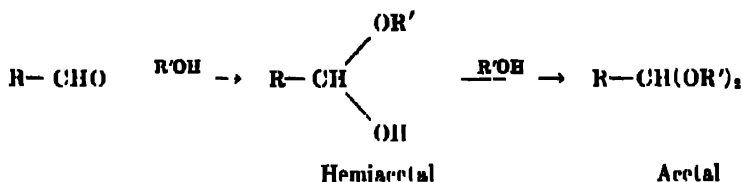
Hemiacetal   Acetal

For the sugar, the hemiacetal (ring) formation takes place by reaction of a hydroxyl with the aldehyde group in the same molecule. Each of the possible ring formulas for glucose allows for two isomers which differ only in the configuration of the hemiacetal group, as carbon 1 is asymmetric in the ring form. Such isomers are distinguished as α- and β-isomers, e.g., α-glucose and β-glucose, and are termed *anomers* The hemiacetal carbon atom sometimes is known as the *anomeric* or *reducing* carbon atom. The existence of isomeric glucoses, pentaacetyl glucoses and methyl glucosides becomes explicable when the sugar and its derivatives have ring structures.

B. Proof of Ring Structure. Subsequent to the proposal of the ring structures for the sugars and derivatives, acceptance by carbohydrate chemists[14] gradually took place. However, it was not until the period 1920 to 1930 that conclusive proof could be offered for the positions of the rings Prior to this work, the rings usually were considered to be of the 1,4 type shown above in the Tollens formula, i.e., with the ring formation between carbons 1 and 4. This type of structure was based mainly on an analogy with the acid series for which it was known that γ-hydroxy acids could be converted to inner esters (lactones) which have the 1,4 or γ-structure.

Methods now are available for the unequivocal determination of the ring structures of the glycosidic derivatives of the sugars. The glycosides are made by condensing the sugars with alcohols in the presence of acids. (For a detailed discussion of the preparation of glycosides and of the details of the determination of the structures, see Chapter V.)

$$\begin{array}{ccc}\boxed{\begin{array}{l}HCOH\\(HCOH)_3\ O\\HC\end{array}} & \xrightarrow{\frac{CH_3OH}{HCl}} & \boxed{\begin{array}{l}HCOCH_3\\(HCOH)_3\ O\\HC\end{array}} + \boxed{\begin{array}{l}CH_2OCH\\(HCOH)_3\ O\\HC\end{array}}\\ H_2COH & & H_2COH \qquad\qquad H_2COH\end{array}$$

---
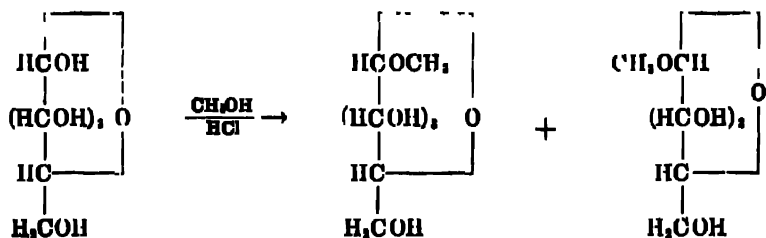
[14] See for example: E. Fischer and K Zach, *Ber* , *45*, 456 (1912), footnote on p. 461.

Originally, the structures of these glycosides were demonstrated by oxidation of the glycosides to fragments which were identified. In order to prevent the oxidation from proceeding too far, the four unsubstituted hydroxyls first were etherified with methyl groups. Details of this method are given later (p. 207). An easier and more direct method involves the periodic acid oxidation of the glycosides. As shown in the formula below, this reagent cleaves the linkage between two adjacent hydroxyl-bearing carbon atoms and removes a hydrogen atom from each carbon. A primary carbinol (CH_2OH) yields formaldehyde; a secondary carbinol ($CHOH$) gives rise to an aldehyde group or, if flanked by two carbinol groups, to formic acid. The reaction is practically quantitative, and the consumption of periodate is a direct measure of the number of adjacent hydroxyl groups in a compound. The structure is determined from the nature of the oxidation products, together with the amount of oxidant that is consumed.

$$
\begin{array}{l}
HCOCH \\
\;| \\
(HCOH)_n \quad\; O \\
\;| \\
HC \\
\;| \\
H_2COH
\end{array}
\xrightarrow[(n>1)]{(n-1)\,HIO_4}
\begin{array}{l}
HCOCH \\
\;| \\
CHO \quad\; O \\
CHO \\
\;| \\
HC \\
\;| \\
H_2COH
\end{array}
+ H_2O + (n-1)\,HIO_3 + (n-2)\,HCOOH
$$
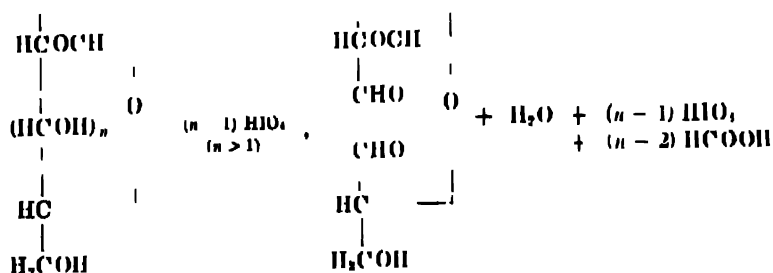
The possible structures for methyl α-D-glucoside are given in formulas I to V. The brackets indicate the adjacent hydroxyl groups.

(I)	(II)	(III)	(IV)	(V)
3	2	2	2	3 Moles oxidant
2	1	0	1	2 Moles HCOOH
1	1	1	0	0 Moles HCHO
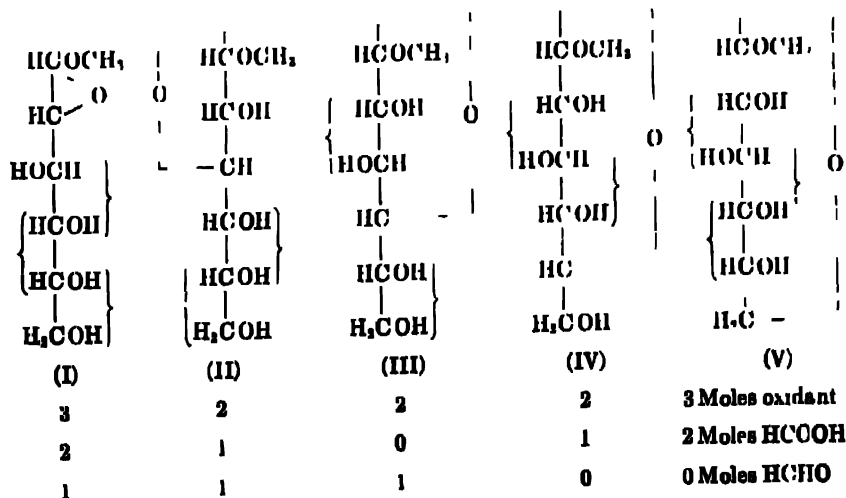
The ordinary methyl glucosides consume two moles of periodic acid, and no formaldehyde is produced. Hence, the structure must be that represented in IV, which has a 1,5 oxygen bridge.

The evidence given above and explained in more detail later (p. 209) confirmed in most instances the structures obtained by the earlier methylation-oxidation studies. The periodic acid oxidation method is used widely because of its simplicity. As a result of the application of the methylation-oxidation technique and of the periodic acid method, it is known that the most common ring present in the glycosides is of the six-membered type connecting carbon atoms 1 and 5. However, rings formed between the 1 and 4 positions are found in some glycosides. Sugars and derivatives which have the 1,5 type of ring may be considered to be derivatives of pyran and those with 1,4 rings to be derivatives of furan. These relations are shown in the accompanying formulas

HCOCH₃	CH	HCOCH₃	CH
HCOH	CH	HCOH	CH
HOCH	CH	HOCH	CH
HCOH	CH	HC	HC
HC	HC	HCOH	
H₂COH		H₂COH	
Methyl glucopyranoside	Pyran	Methyl glucofuranoside	Furan
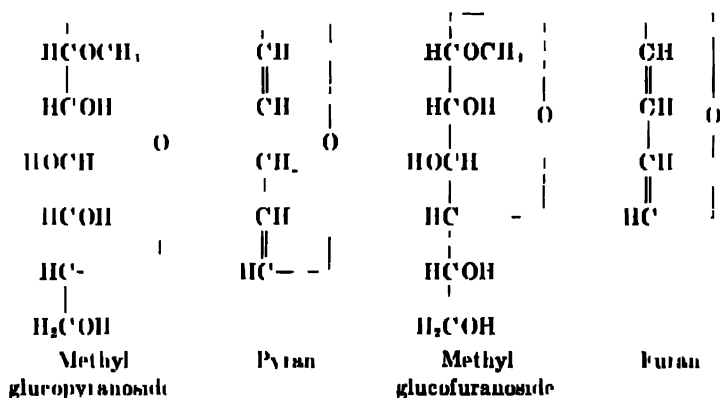
The sugars related to pyran are known as pyranoses, and the corresponding glycosides as pyranosides. Those with furan rings are furanoses and furanosides, respectively.

Although absolute methods are available for the establishment of the ring structures of the glycosides, the corresponding methods for the sugars are indirect. For the glycosides, the rings usually are quite stable under alkaline and neutral conditions. However, in the case of the sugars, difficulties arise from the ease with which ring changes may take place as soon as dissolution of the sugar occurs. The methods which are applicable to the determination of the ring forms of the sugars must be such that ring changes do not precede the necessary reactions. In the following methods, this condition is assumed.

One method for locating the position of the ring in unsubstituted sugars requires oxidation to the corresponding acids or lactones. As shown in the following formulas, the ring compounds should be oxidized (dehydro-

genated) by bromine to the corresponding lactones. whereas the free alde-
hyde forms would give the corresponding acids.

$$
\begin{array}{cccc}
\text{HCOH} & \text{OC} & \text{OC} & \text{HCOH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{HOCH} \quad O & \text{HOCH} \quad O & \text{HOCH} & \text{HOCH} \quad O \\
\text{HCOH} & \text{HCOH} & \text{HC}\!-\!- & \text{HC}\!-\! \\
\text{HC}\,-\,- & \text{HC}\,-\, & \text{HCOH} & \text{HCOH} \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{H}_2\text{COH} & \text{CH}_2\text{OH}
\end{array}
$$

Glucopyranose 　　Gluconic 　　　Gluconic 　　　Glucofuranose
　　　　　　　　　δ-lactone 　　　γ lactone

$$
\begin{array}{cc}
\text{OCH} & \text{COOH} \\
\text{HCOH} & \text{HCOH} \\
\text{HOCH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} \\
\text{HCOH} & \text{HCOH} \\
\text{H}_2\text{COH} & \text{H}_2\text{COH}
\end{array}
$$

　　　Gluconic aldehyde 　　　　　Gluconic acid
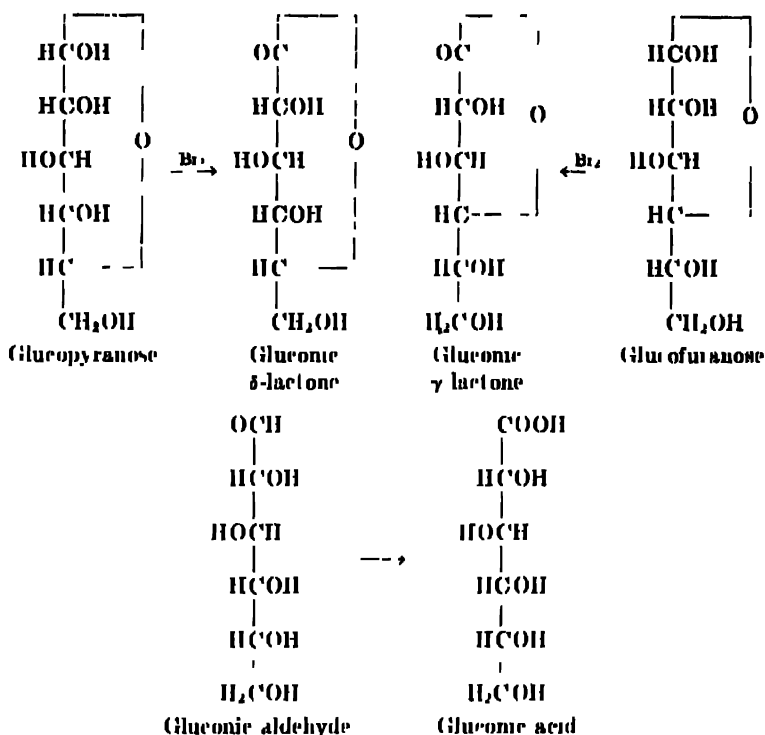
The oxidation reaction takes place in solution, and the nature of the
oxidation products establishes the structure of the original sugar unless
ring shifts take place prior to the oxidation reaction. By application of this
method[16], it has been shown that the common form of D-glucose (the alpha
isomer) gives gluconic δ-lactone The β-D-glucose gives the same material.
Hence, both have pyranose (1,5) rings, otherwise the γ-lactones or the
free acids would be produced. The method has not been widely applied. A
crystalline addition compound of mannose and calcium chloride yields
mannonic γ-lactone, and appears to have a furanose structure[16].

By the bromine oxidation method, the structure of the sugars can be
correlated with those of the corresponding lactones and acids. The proof
requires that the structures of the lactones be known. In general, the
method depends on a correlation of the properties of the lactones with those

[16] H S Isbell and W W Pigman, *J Research Natl Bur Standards*, 10, 337 (1933),
H S Isbell and C S Hudson, *ibid*, 8, 327 (1932), H S Isbell, *ibid*, 9, 615 (1932)
[16] H S Isbell, *J Am Chem Soc*, 55, 2166 (1933)

of the methylated derivatives obtained by methylation and oxidation of the glycosides of known structures.

Another method for the establishment of the ring structures of glucose (and other sugars) involves the correlation of the optical rotations of the sugars with those of the glycosides. This method, although not absolute, was developed and widely applied by C. S. Hudson and has much value for this purpose. It is considered in a later section (p. 80).

The glucosides are hydrolyzed to glucose by certain enzymes (see Chapter XI). The identification of the form of the sugar which is released provides a method for the correlation of glycosides with the crystalline forms of the sugar[17]. The product formed by the enzymic hydrolysis of methyl α-glucoside appears to be the ordinary α-isomer; that from methyl β-glucoside appears to be the β-isomer. Hence, unless ring changes take place very rapidly, the α- and β-forms of glucose would appear to have the same pyranose structures as the corresponding glucosides.

The present methods for the determination of the structures of the unsubstituted sugars are rather unsatisfactory as absolute methods because of the possibility of ring shifts. However, the evidence which is available indicates that most of the crystalline sugars have pyranose ring structures. A double compound of mannose with calcium chloride probably has the furanose structure[16] and a disaccharide ketose, lactulose, may exist as the furanose modification when in the crystalline state[18]. Otherwise crystalline furanose derivatives are known positively to exist only in compounds in which ring shifts are not possible (glycosides, disaccharides, etc.) or in compounds in which the hydroxyl that forms the pyranose ring is blocked by substitution with a stable group.

C. Configuration of the Anomeric Carbon Atom. For each of the ring modifications of the sugars, two isomers can exist, because a new asymmetric carbon atom is created by ring closure at the reducing carbon atom. These isomers are known as α,β-isomers or anomers.
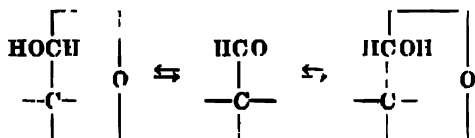
As noted previously, the existence of such isomers was one of the most important reasons for the formulation of ring structures. The isomeric α- and β-glucoses have quite different solubilities, melting points and rotations. The isomeric pentaacetates and methyl glucosides exhibit similar differences in properties.

The conductivity of sugars freshly dissolved in boric acid solution may give an indication of the absolute configuration of the anomeric carbon

[17] E. F. Armstrong, J. Chem. Soc., 83, 1305 (1903)
[18] H. S. Isbell and W. W. Pigman, J. Research Natl. Bur. Standards, 20, 773 (1938).

atom.[19, 20a] Boric acid forms complexes, probably of an ester structure, with *cis* hydroxyl groups on neighboring carbon atoms (see p. 183 and 253). When α-glucose is dissolved in a boric acid solution, the conductivity of the solution decreases with time until a constant value is reached; on the ot' er hand, the conductivity of β-glucose solutions increases with time. This behavior would be expected for these sugars, if α-glucopyranose has a *cis* pair of hydroxyls at carbon atoms 1 and 2, and β-glucopyranose a *trans* pair.

The above evidence conforms with the accepted configurations for carbon one of the anomeric D-glucoses and played an important part in the acceptance of these configurations. However, Boeseken and his co-workers have shown that when adjacent *cis* hydroxyl groups are present in a strainless six-membered ring, boric acid may not react because of the mutual repulsion of such groups, i.e., the adjacent hydroxyl groups will tend to be oriented as far apart as possible. Also, Hückel and co-workers[20b] have shown that the geometry of six-membered carbon rings of the strainless type is such that *cis* groups may be oriented a maximum of 72° apart, whereas *trans* groups may approach as close as 18° Additional complications arise, for most sugars other than glucose, in that pairs of contiguous *cis* hydroxyls are present in addition to those at carbons 1 and 2. Also, the furanose form may react preferentially.[20c]

The periodic acid oxidation provides a means for correlating the configuration of the anomeric carbon atoms of the glycosides (see also p. 210). As shown in the accompanying formulas, representative of the hexosides, carbon 3 is removed in the process (as formic acid), and the asymmetry of carbons 2 and 4 is destroyed.

1	HCOCH₃		HCOCH₃	
2	HCOH		HCO	
3	HOCH	O		O
		$\xrightarrow{HIO_4}$ HCOOH +		
4	HCOH		HCO	
5	HC—		HC-	
6	H₂COH		H₂COH	

Methyl α-D-glucoside
(IV)   (V)
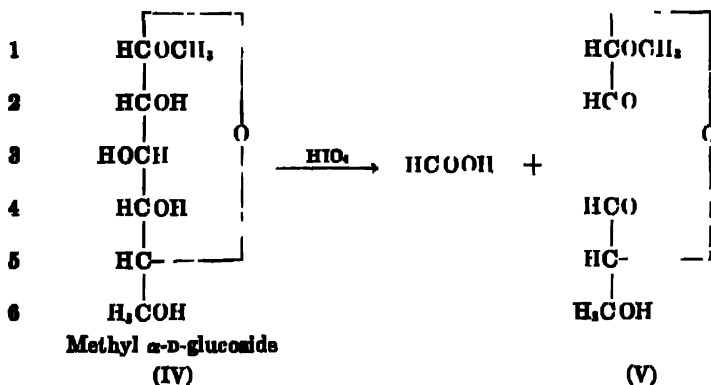
In the dialdehyde (V) only two asymmetric carbon atoms remain, and these are derived from carbon atoms 1 and 5 of the original glucoside (IV).

[19] For summary see. J Böeseken and H. Couvert, *Rec. trav chim* , 40, 354 (1921)

[20a] R. Verschuur, *Rec. trav. chim.*, 47, 123, 423 (1928).

[20b] W. Hückel and coworkers, *Ann.*, 533, 128 (1937); *Chem. Abst.*, 32, 3373 (1938).

[20c] See: J Böeseken, *Rec. trav. chim.*, 61, 663 (1912); *Chem. Abst.*, 39, 2054 (1945)

Hence, all of the D-aldohexosides should yield the same dialdehyde (V) as the corresponding α- or β-D-glucosides. The configuration of carbon 1 of each of the glycosidic derivatives of the hexoses may be correlated with those of the glucosides in this manner.[31]

The method employing periodic acid oxidation does not allow for an absolute determination of the configuration of the hemiacetal carbon atom of the glycosides, but it provides a method by which the configuration of the hemiacetal carbon of the various hexosides may be correlated with that of the glucosides. By means of comparisons of optical rotation or by a study of the products of enzymic hydrolysis, the relation between the configuration for carbon atom 1 of the glucosides and glucose may be established. However, the development of more absolute methods would be a very desirable undertaking. Such methods are needed particularly for the ketoses.

Additional evidence for the configuration of carbon 1 of some of the phenyl glycosides has been obtained[32] from studies of the action of alkalies (p. 215). The β-isomers form a 1,6-anhydro ring by the elimination of phenol whereas the α-isomers are affected only slowly or not at all. Unless a Walden inversion takes place in the formation of the anhydro ring, it would be expected that the reaction would take place between groups having a cis relationship with respect to the sugar ring. Hence, the reactivity of the β-glycosides might be ascribed to a cis relation between the groups involved, i.e., the phenyl group at carbon 1 and the primary hydroxyl group at carbon 6.

D. The Representation of the Ring Structures of the Sugars. In the preceding discussion, the structure and configurations of the two isomeric glucoses have been developed. The structure and configuration may be represented by the cyclic form of the Fischer formula as in I for α-glucose and as in II for methyl α-glucoside.

| (I) | (II) | (III) |
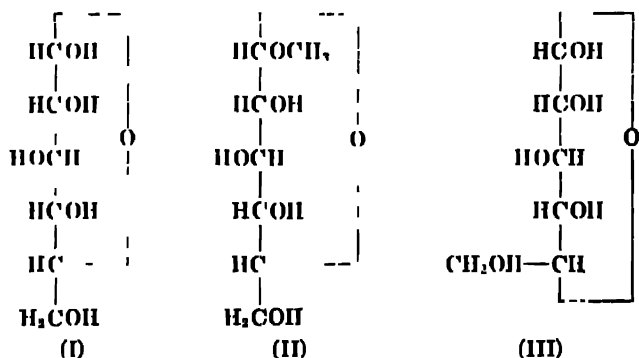
[31] E. L. Jackson and C. S Hudson, *J. Am. Chem. Soc.*, **59**, 994 (1937)

[32] E. M Montgomery, N K Richtmyer and C S Hudson, *J. Am. Chem. Soc.*, **65**, 3 (1943)

However, the cyclic, Fischer-Tollens formula has several shortcomings. Thus, the molecule is represented as an extended chain of carbon atoms connected by an oxygen bridge between positions 1 and 5. Obviously an extended linear chain is impossible, for carbon atoms 1 and 5 must be close enough for the existence of the oxygen bridge. The configuration of carbon atom 5 as given by the cyclic Fischer formula also does not give a correct picture of the steric relations between the terminal primary hydroxyl group and the hydroxyl groups attached to the ring carbon atoms. A formula of the type of III would give a more correct representation of the configuration of carbon atom 5; thus, the primary hydroxyl group is shown to have a *trans* relationship to the hydroxyl groups at carbon atoms 1, 2 and 4.

In order to provide a better picture of the structure and configuration of the glucose molecule, Haworth has proposed a perspective representation. That for α-glucose is shown in formula IV.

(IV)   (V)   (VI)
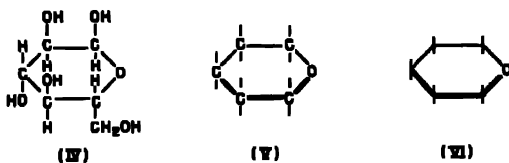
The Haworth formula is to be considered as a conventionalized perspective drawing of a three-dimensional model. The basic pyranose ring is represented in V and VI as a ring in which all of the atoms lie in a single plane. The formulas IV, V and VI are to be considered as projections of a hexagonal heterocyclic ring. The hexagon is held so that the observer looks from above; its nearest edge appears as the bottom lines in the above formulas. The edge closest to the observer appears as heavy black lines in V and VI. In formula V, the valences projecting above the plane are equivalent to a position to the right in the Fischer formula.

In the present volume, a modified form of the Haworth formula will be used in order that an easier transposition from the Fischer to the Haworth formulas will be possible. The transposition from the Fischer to the Haworth type of formula is illustrated below in formulas VII, VIII and IX. The Haworth formula IX is formed from VIII by ring closure between carbon atoms 1 and 5. Formula IX may be further simplified as in X and XI by representing the pyranose ring as a hexagon with an oxygen atom at one corner. The side of the ring closest to the observer can be

indicated by heavy lines as in X although this shading frequently is omitted as in XI.

(HO)H_2C—C—C—C—CHO

(VII)

(VIII)

(IX)

(X)

(XI)
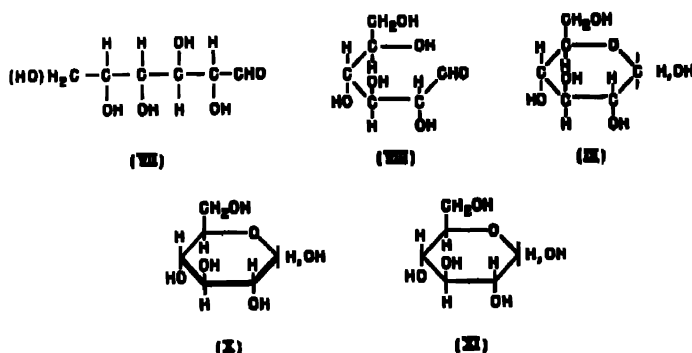
The configurations of the asymmetric carbon atoms in the Haworth formula of the type of IX, X and XI may be related easily to those in the corresponding Fischer formula VII. Thus, it can be seen that the hydrogen atoms and hydroxyl groups on carbon atoms 2, 3 and 4 are represented in the same fashion in both types of formulas. The configuration of carbon atom 1, although not represented in the aldehyde structure, is written in the same manner in the ring form of the Fischer formula and in the particular form of the Haworth formula used here. As shown in VIII, the primary alcoholic group projects above when the hydroxyl group of carbon 5 lies to the right in the Fischer formula. In the D-series of the aldohexopyranoses, the terminal primary hydroxyl group projects above the plane of the ring atoms; in the L-series, it lies below. When the ring is viewed from the opposite side of the model, as in IV, the configuration of each of the carbon atoms is represented in the opposite manner from that in IX, X and XI.

Frequently it may be desirable to orient the ring in positions other than that shown in IX, X and XI. This may be particularly important when bulky groups are present or when linkages between two or more rings are to be represented as for the oligo- and poly-saccharides. (Two of the possibilities are given later for each of the pentoses and hexoses, see p. 57.) Since the Haworth formulas are not projections of the Fischer type, they cannot be rotated in the plane of the paper. Reorientations must take place in space.

When the "tail" group attached to the ring contains an asymmetric carbon atom as in the heptopyranoses or the hexofuranoses and the corresponding glycosides, a convention is necessary to represent the configuration of this asymmetric atom. To do this, the Fischer convention may be applied for the "tail" group. Thus, glucofuranose would be:

HCOH
HCOH
HOCH
HC
HCOH
H₂COH

(XII)

H₂COH
HOCH
CH
HCOH
HOCH
HOCH
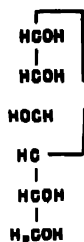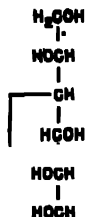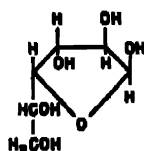
(XIII)
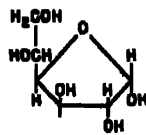
The Haworth type of formula represents a considerable improvement over the older cyclic forms of the Fischer formula. Thus, for such formulas it is much clearer that substances might react simultaneously with groups at carbon atoms 4 and 6 or at 1 and 6 to form bridges between these positions. The Haworth formulas, however, are in turn only approximations of the molecular structures. The placement of all atoms in a single plane undoubtedly is an over-simplification. In a single coplanar ring, the valence angles necessarily would be appreciably greater than those in a "strainless" structure having valence angles of 109°.

As pointed out by Haworth,[23] a number of stereoisomeric structures are possible for the pyranose ring of each sugar, and at least ten types are capable of existence.[24] Several of the possible structures are illustrated in Fig. 1. As shown in I and II, four of the atoms of the ring may lie in one plane, and the other two atoms may lie on the same side of the plane as in II. The structure represented in II is the cis or boat type; that in I is the trans or chair type of structure. The "strained" coplanar type of structure is represented in V. The structures represented in III and IV have five ring-atoms in one plane and the sixth atom projects below the plane of the other atoms. According to the X-ray studies by Cox and associates,[25] structures IV and V may represent best those of the crystalline sugars. It would be expected that in solutions the energy difference between the various isomers would be so small that all forms would exist in the equilibrium solution.

Rings containing seven or more atoms are inescapably puckered and cannot be represented properly in the planar Haworth formulas. In drawing multi-ring compounds, such as 1,6-dibenzoyl-2,5-methylene-3,4-benzylidene-D-mannitol, the best solution probably is to select the most rigid ring as the principal ring for projection and to adjust the more flexible large ring to the smaller ring. The mannitol derivative is shown below in the Fischer-Tollens projection (XIV), in a planar Haworth-type formula (XV) and

[23] W. N. Haworth "The Constitution of Sugars," p 90, Edward Arnold & Co, London (1929)

[24] O. L. Sponsler and W. H. Dore, Ann Rev Biochem, 5, 66 (1936)

[25] E. G. Cox, T H Goodwin and A. I. Wagstaff, J Chem Soc (1935, 978, 1495
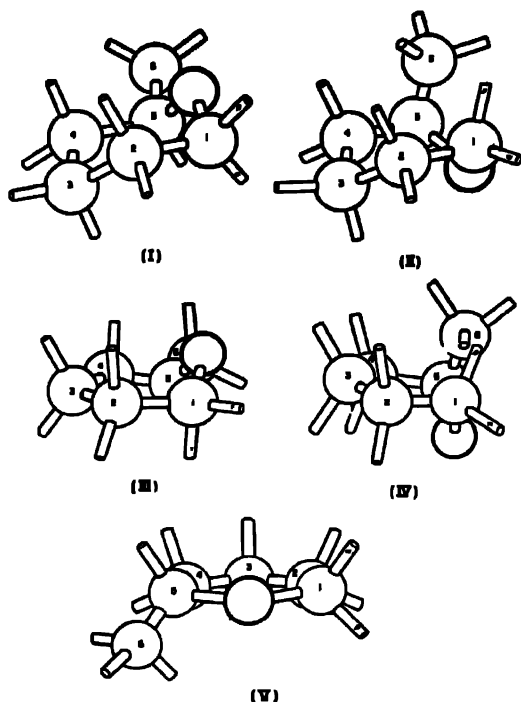
Fig. 1. Strainless ring stereoisomers of the pyranose ring (I to IV).
The heaviest circle indicates the oxygen atom.
(Reprinted from: *J. Research Natl. Bur. Standards*, **18**, 522, (1937)).

as a perspective sketch (XVI). A study of models is essential for arriving at the best method for the presentation of multi-ringed compounds.
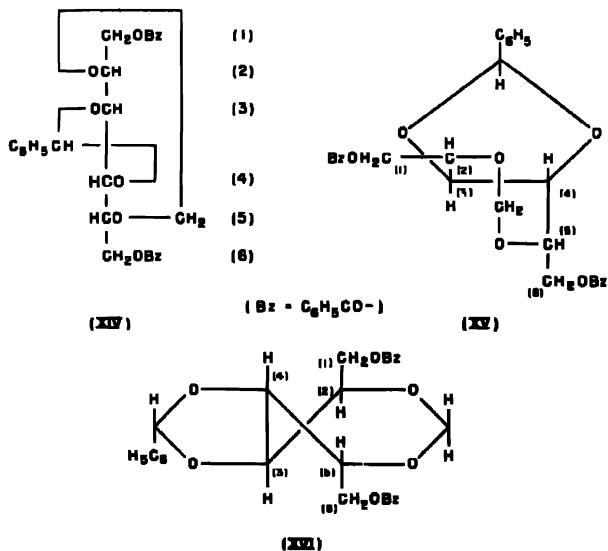
Note that the Fischer convention does not apply to XV and XVI which are space models. Since there is no convention established for representing the configuration of atoms in the secondary ring of XV, the representation must be considered as arbitrary.

E. Nomenclature of Anomers (α-β **Nomenclature**). The system of nomenclature for the anomeric (α, β) isomers of the sugars most commonly employed is that of C. S. Hudson.[26] According to this system, for sugars of the D-series the more dextrorotatory isomer of each α-β pair is known as the alpha isomer; the lesser dextrorotatory isomer is the beta isomer. For sugars of the L-series, the converse is true. A correct application of the system requires knowledge that the compounds being considered are truly anomeric, i.e., that they differ only in the configuration of the hemiacetal carbon atoms. If the compounds being considered mutarotate and are of the D-series, usually the α-isomer is the form which mutarotates to a value less positive than the initial value. Particularly in the case of compounds which exhibit complex mutarotations, the mutarotation data must be interpreted with caution, for it can be used for the naming of the sugars only when it represents an α- to β-interconversion.

The structural significance of the rules has been explained by Hudson[27] who writes the skeleton stereostructures for the methyl α- and β-pyranosides as follows:

α D \
β L ⎰ Methyl glycosides
(Partial rotation of C₁ is *dextro* in sign)

β D \
α L ⎰ Methyl glycosides
(Partial rotation of C₁ is *levo* in sign)
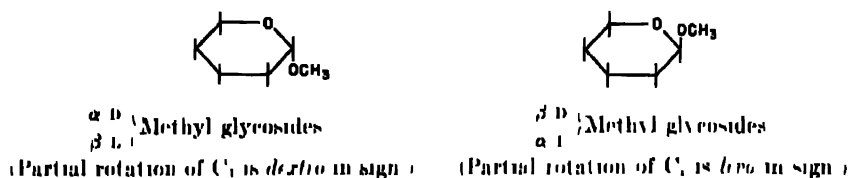
The skeleton formulas for the anomeric furanose forms are:

α D \
β L ⎰ Methyl glycosides
(C₁ is dextrorotatory)

β D \
α L ⎰ Methyl glycosides
(C₁ is levorotatory)
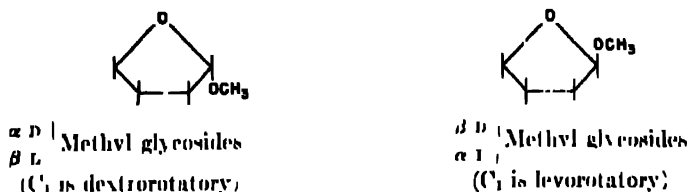
26 a. C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

b. For further discussion and other systems see. H. S. Isbell, *J. Research Natl. Bur. Standards*, **18**, 505 (1937).

c. N. A. Sørensen, *Kgl. Norske Videnskab. Selskabs Forh.*, no. 2 (1937); C. N. Riiber and N. A. Sørensen, *ibid.*, no. 1 (1938). The term "anomers" originated with the latter workers

27 C. S. Hudson, *J. Am. Chem. Soc.*, **60**, 1537 (1938). The Haworth type formulas which are given have been turned over 180° in space to be in conformity with the formulas as written in the present work. See previous discussion

For the pyranosides of the D aldohexoses and higher-carbon sugars of the D-series, carbon atom 6 is written as projecting above the pyranose ring when written as above; for the corresponding derivatives of the L series, carbon atom 6 lies below the pyranose ring (see discussion earlier in this chapter). The same rule applies to the aldofuranosides except that it is carbon atom 5 which lies above the ring in the D series and below the plane in the L series; also, the rule may be applied to the aldo pentofuranoses

It should be noted that α D and β L (also β D and α L) refer to the same absolute configuration of the anomeric carbon atom Thus, β L arabinose and α-D-galactose have the same absolute configuration at carbon atom 1 and at the other asymmetric atoms and hence exhibit many similarities

Anomeric modifications of open-chain derivatives of sugars have been prepared. Thus, two 1-chloro-*aldehydo*-D-galactose hexaacetates (XVII and XVIII) are known.[28]

<pre>
        H   H
        | |
     ClCOAc   AcOCCl
        | |
     HCOAc   HCOAc
        | |
     AcOCH   AcOCH
        | |
     AcOCH   AcOCH
        | |
     HCOAc   HCOAc
        | |
     CH₂OAc   CH₂OAc
      (XVII)   (XVIII)
</pre>
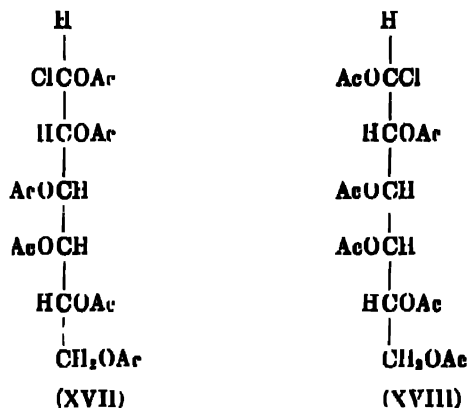
These modifications, in analogy to the cyclic forms, have been named α and β according to whether they mutarotate downwards (α) or upwards (β) in acetyl chloride solution containing zinc chloride.[29]

4. Homomorphous Sugars

A. Homomorphology. Earlier in this chapter, the genetic relationship of the various sugars to D- and L-glyceraldehyde was demonstrated. A relation of considerably more importance for the correlation of the properties and reactions is based on the similarity of substances which have the same configurations for the atoms which compose the pyranose rings. Since for

[28] M. L. Wolfrom and R. L. Brown, *J. Am. Chem. Soc.*, *63*, 1246 (1941).

[29] For a further discussion of the naming of this type of compound see: M. L. Wolfrom, M Konigsberg and F. B. Moody, *J Am Chem Soc*, *63*, 2343 (1940); R. J Dimler and K P. Link, *ibid.*, *62*, 1216 (1940).

the aldohexoses the number of asymmetric carbons is just sufficient to make each carbon atom in the pyranose ring asymmetric, the aldohexoses may be considered as the basic types for all sugars which can form pyranose rings. The pentoses and higher sugars can be obtained from the hexoses by substitution of the —CH₂OH groups of the hexoses by H or by (CHOH)ₙ-- CH₂OH, respectively. The various hexose types are illustrated in the accompanying formulas which also show some of the members of each series. Although 32 hexopyranoses theoretically are possible, only the formulas for the eight D-types are written and the α,β configuration is not indicated. Because of the lack of asymmetry of carbon atom 5 of the aldopentoses, each of these sugars is related to a pair of hexoses. On the other hand, the basic types of the furanoses are the pentoses.

Homomorphous Series of Pyranoses and Furanoses

D-Idose type

X = - H, L-Xylose

X = --CH₂OH, D-Idose

X = —CH₃, 6-Desoxy-D-idose

X = --CHOH--CH₂OH, { D-Gluco-D-*ido*-heptose L-Ido-D-*ido* heptose

D-Gulose type

X = —H, L-Lyxose

X = —CH₂OH, D-Gulose

X = —CH₃, 6-Desoxy-D-gulose

X = —CHOH—CH₂OH, { D-Gluco-D-*gulo* heptose L-Ido-D-*gulo* heptose
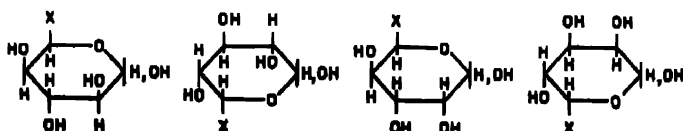
D-Glucose type

X = H, D-Xylose

X = CH₂OH, D-Glucose

X = CH₃, D-Isorhamnose

X = —CHOH—CH₂OH, { L-Gala-D-*gluco*-heptose D-Altro-D-*gluco*-heptose
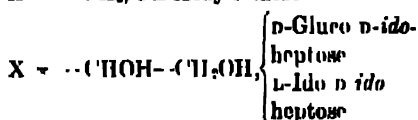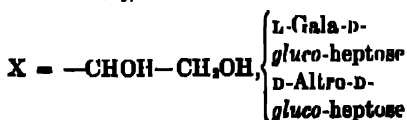
D-Mannose type

X = H, D-Lyxose

X = —CH₂OH, D-Mannose

X = - CH₃, D-Rhamnose

X = —CHOH—CH₂OH, { D-Altro-D-*manno*-heptose L-Gala-D-*manno*-heptose

D Galactose type
X = -H, L Arabinose
X = CH₂OH, D Galactose
X = CH₃, D Fucose

X = CHOH CH₂OH, { D Manno D gala-heptose / L Gulo D gala heptose

D Talose type
X = H, L Ribose
X = CH₂OH, D Talose
X = CH₃, 6-Desoxy D talose

X = CHOH CH₂OH, { D Manno D talo heptose / L Gulo D talo heptose

D Allose type
X = H, D Ribose
X = CH₂OH, D Allose
X = CH₃, 6 Desoxy D-allose

X = —CHOH CH₂OH, { D Allo D allo heptose / L Talo D allo heptose

D Altrose type
X = H, D Arabinose
X = CH₂OH D Altrose
X = CH₃ 6 Desoxy D altrose

X = CHOH CH₂OH, { D Allo D altro heptose / L Talo D altro heptose

D Xylose type
X = H, L Threose
X = CH₂OH, D Xylose

X = CHOH CH₂OH, { D Glucose / L Idose

D Lyxose type
X = H L Erythrose
X = CH₂OH, D Lyxose

X = CHOH CH₂OH, { D Mannose / L Gulose

D Arabinose type
X = H, D Threose
X = CH₂OH, D Arabinose
X = —CHOH —CH₂OH, { D Altrose / L Galactose

D Ribose type
X = H, D-Erythrose
X = CH₂OH, D Ribose
X = CHOH CH₂OH { D Allose / L Talose

As would be expected from the identity of the configurations of the pyranose or furanose rings, the members of each homomorphous series

show marked chemical and physical similarities[30], and it is often possible to predict the properties of unknown members from those of the basic type. The greatest differences, as might be anticipated, are found between the pentoses and the corresponding hexoses.

Extensive correlations of properties of the members of each hexose series have not been carried out, but the value of the concept may be illustrated by the following instances. Thus, the members of the mannose series are noted for the ease with which orthoesters are formed when acylglycosyl halides react with alcohols in the presence of silver carbonate (page 193).

Tetraacetylglucosyl bromide

Methyl β glucoside tetraacetate

Tetraacetylmannosyl bromide

Triacetylmannose methyl 1,2 orthoacetate
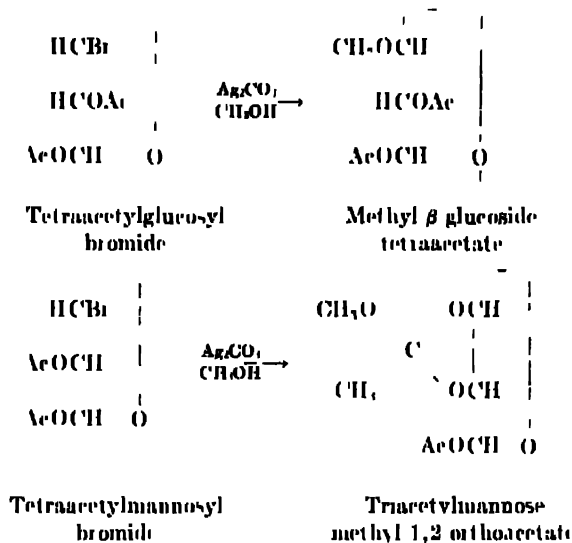
The β-glucoside and its homomorphs are obtained in this manner from the members of the glucose series, but the β-mannoside and its homomorphs are obtained only by other means. The members of the galactose series exhibit mutarotations which are complex in character and which may pass through a maximum or minimum; in contrast, the mutarotations of the members of the glucose series follow the first-order equation.[31] Members of the gulose and mannose series form crystalline calcium chloride addition products more readily than those of the glucose series. In fact, the only known crystalline form of gulose is its calcium chloride compound. Although additional experimental work is necessary to confirm the conclusion, it appears that enzymes which hydrolyze the hexoside members of each

[30] R. M. Hann, A. T. Merrill and C. S. Hudson, *J. Am. Chem. Soc.*, **57**, 2100 (1935); R. M. Hann and C. S. Hudson, *ibid.*, **59**, 548 (1937); H. S. Isbell, *J. Research Natl. Bur. Standards*, **18**, 505 (1937); H. S. Isbell and W. W. Pigman, *ibid.*, **18**, 141 (1937). Many earlier workers had also noticed the resemblances in the structures for the members of the various series

[31] H. S. Isbell, *J. Research Natl. Bur. Standards*, **18**, 515 (1937); **20**, 97 (1938)

series also hydrolyze the glycosides of the other members of each series.[22]
*Thus, the enzyme α-mannosidase of almond emulsin probably hydrolyzes
the α-lyxosides as well as the α-mannosides.*

The optical rotations of the members of each series and of their deriva-
tives often exhibit marked similarities. The rotations of derivatives of D-
mannose and L-gala-D-*manno*-heptose are compared in Table II. (The
rotations actually recorded in the literature are those for the enantio-
morphous form of the heptose; the sign of rotation has been changed in the
following list.) Riiber and Sørensen have shown that the rates of muta-

TABLE II

Comparison of the Rotations of Two Homomorphous Sugars and Their Derivatives[24]

Type derivatives	D Mannose derivatives	L Gala-D manno-heptose derivatives
Free sugar (initial)	+34	+26
Free sugar (final)	+14 6	+15.3
Sodium salt of aldonic acid	8 8	−9.6
γ Lactone	+51.8	+52 3
Aldonic acid phenylhydrazide	8.1	−8.5
Aldonic acid amide	−17 3	−14.3
Methyl glycoside	+79 2	+70.2
Methyl glycoside acetate	+49 1	+20.4
Benzyl mercaptal	32.9	30 3

rotation are characteristic for each homomorphous series[26] Thus the
members of the glucose series mutarotate more slowly than the corre-
sponding members of the galactose and mannose series.

The ketoses also may be considered to belong to the aldose homomorph-
ous series as is shown by a comparison of the formulas for D-arabino-
furanose and D-fructofuranose
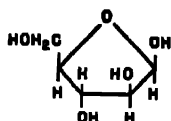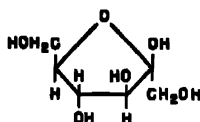
β D Arabinofuranose   β D Fructofuranose

These two sugars would be expected to exhibit many similar properties
and reactions. However, considerable differences might arise from the
replacement of the hydrogen atom of the aldose by the CH_2OH group of
the ketose.

B. Nomenclature of the Higher Sugars. The customary method of
naming sugars which have carbon chains longer than those in the hexoses

[22] W. W. Pigman, *J Research Natl. Bur Standards*, *26*, 197 (1941)
[24] R M Hann, A. T Merrill and C. S. Hudson, *J Am Chem Soc.*, *57*, 2100 (1935)

has been to relate the higher sugars to the hexose from which they might
be derived and to add an ending denoting the number of carbon atoms in
the molecule. Thus, there are glucoheptoses, mannooctoses, gluconononoses,
etc. The two stereoisomeric D-glucoheptoses have been distinguished by th(
prefixes α- and β-. The D-α-glucoheptose was the sugar obtained by Emil
Fischer by the application of the cyanohydrin synthesis to glucose. Its
2-epimer is D-β-glucoheptose. In the early work, the configuration of the
asymmetric carbon atom at the second carbon atom was unknown, and the
α- and β- were applied according to the order of isolation. The α- designation
was given to the first known isomer. A configurational basis for the appli-
cation of α- and β- has been suggested by Isbell.[1a]

Hudson[1b] has suggested that a prefix denoting the configuration of the
first four asymmetric centers be introduced into the name instead of using
the Greek letters. This portion of the name is placed in italic to show that
it relates only to configuration. This system has the advantage that it
retains the elements of the older name and yet indicates in the italicized
portion the homomorphous series to which the substances belong. The
position of the configurational portion is different from that in names such
as *xylo*-trimethoxyglutaric acid. The application of the system to the two
glucoheptoses is shown in the accompanying formulas

| | | D-Glucose | D Gluco-D-*gulo* heptose (D-α- Glucoheptose) | D-Gluco-D-*ido* heptose (D-β- Glucoheptose) |
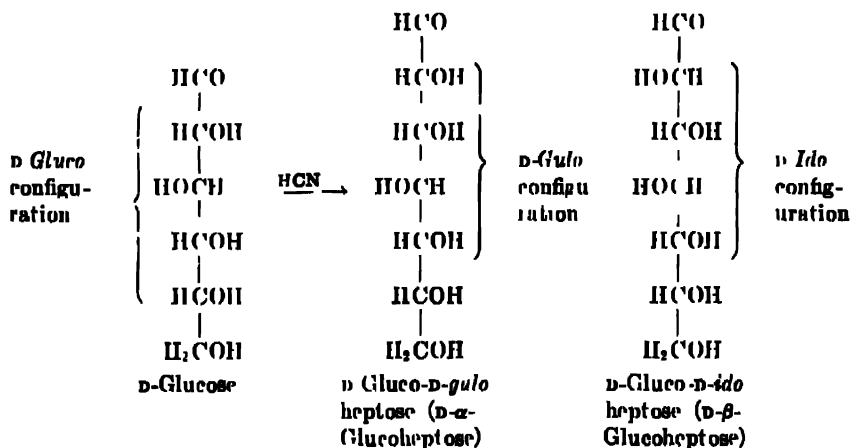
It should be noted that if there is any ambiguity, the entire configuration can be
described (as in D gluco-D-*glycero* D *gulo*-heptose or D *glycero* D *gulo*-heptose) by the
introduction of a term such as D *glycero* or D *threo* to indicate the configuration of
the asymmetric center or centers not covered by the D-*hexo*-term.[1c] Ordinarily, it

[1a] H. S. Isbell, *J. Research Natl. Bur. Standards, 18*, 529 (footnote) (1937).

[1b] C S. Hudson, *J. Am Chem Soc , 60*, 1537 (1938); *Advances in Carbohydrate Chem , 1*, 28 (1945).

[1c] For examples of the complete definition of a sequence of asymmetric carbon atoms see:

would not be necessary to be completely specific since the unitalicized hexo term defines the compound.

The heptuloses and higher ketoses can be named[33d] by using as the italicized portion the name of the corresponding ketose. Thus, D-gala-heptulose could be termed D-gala-L-*fructo*-heptose as shown in the formula. For the heptuloses, this is not necessary for the ordinary name is completely definitive.

1 -*fructo*

The α,β nomenclature of the heptoses usually has been based on the unitalicized portion of the name. However, it seems better in most instances to base it on the italicized portions because the hexoses and heptoses of the same homomorphous series are given the same name for the same configuration of the anomeric carbon atom. α-D α-Galaheptose·

could be α-D-gala-L-*manno*-heptose, or D-gala-β-L-*manno*-heptose.

5. The Sugars in Solution

A. In the Absence of Strong Acids or Alkalies; Mutarotation. Although fairly stable when in the crystalline condition, the sugars undergo many transformations when dissolved in water, particularly in the presence of acids or alkalies. Initially, these changes usually involve the carbon atom carrying the aldehyde or ketone groups. Hence, when these groups are blocked as in the non-reducing compound sugars (*e.g.*, sucrose) or glycosides, the compounds are more stable and do not undergo isomerizations until the blocking groups are removed.

E. F. Degering, "*An Outline of the Chemistry of the Carbohydrates*," p 261 and 265; John S. Swift Co , Cincinnati, Ohio (1913).

H. S Isbell, *J. Research Natl. Bur. Standards*, **34**, 111 (1945), footnote 3 Also note comments by C. S. Hudson in footnotes in reference 33b.

[33d] M. L. Wolfrom, R L. Brown and E F. Evans, *J Am Chem Soc.*, **65**, 1021 (1943)

Interconversions between α,β isomers and between ring isomers take place under the mildest possible condition of acidity and temperature. Such changes are manifested by the change of optical rotation with time which may be observed for freshly prepared sugar solutions. The change of optical rotation with time is known as mutarotation. Mutarotations may arise from changes other than interconversions between α,β and ring isomers, but for neutral or slightly acid or slightly alkaline solutions of the sugars, they usually arise from such changes. The phenomenon was observed first by Dubrunfaut (1846) who noted that the optical rotation of freshly dissolved glucose changes with time and that after a number of hours, the rotation becomes constant. The ordinary form of glucose (α-glucose) mutarotates downward, and the β-isomer mutarotates upwards; in both cases the same equilibrium value is reached.

$$\alpha\text{-Glucose} \rightleftarrows \text{equilibrium} \rightleftarrows \beta\text{-Glucose}$$
$$+112^\circ \quad \longrightarrow \; + 52 7^\circ \; \longleftarrow \quad + 18 7^\circ$$
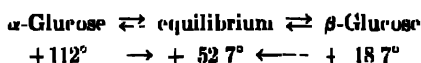
As mentioned earlier in this chapter, the mutarotation of glucose and other sugars showed that the original aldehyde structure for glucose was not adequate for explaining the properties of the sugar. The separation of several isomers of lactose (Erdmann -1880) and of glucose (Tanret--1896) which mutarotated to the same equilibrium value provided good evidence that the observed mutarotations result from an interconversion of the various modifications.

The mutarotation of α-glucose may be represented by the equation for a first-order reversible reaction.

$$\alpha \underset{k_2}{\overset{k_1}{\rightleftarrows}} \beta \tag{I}$$

$$\frac{d\alpha}{dt} = k_1|\alpha| - k_2 |\beta| \tag{II}$$

Equation (II) gives the rate of change of the α- into the β-form at the time t. The reaction constant for $\alpha \rightarrow \beta$ is k_1, and for $\beta \rightarrow \alpha$ is k_2. The concentrations of the alpha and beta form at the time t are represented by $|\alpha|$ and $|\beta|$.

As shown by Hudson, the equation (II) may be integrated and expressed in terms of the optical rotations in the form of equation (III).

$$k_1 + k_2 = \frac{1}{t} \log \frac{r_0 - r_\infty}{r_t - r_\infty} \tag{III}$$

In equation (III), r_0 = the rotation at $t = 0$, r_∞ = the final equilibrium rotation, and r_t = the rotation at the time t. The rotations may be expressed as observed or specific rotations. The specific rotations are calculated from the observed rotations (α) by the relation

$$|\alpha| = \frac{\alpha \times 100}{l \times c} \tag{IV}$$

α = observed rotation.

l = length of column of solution (expressed in decimeters).

c = concentration of active substance as g./100 ml. of solution

In case the rotations are read on a saccharimeter, the values observed (°S) are multiplied by the factor 0.3462 to give α

The rotation varies with the wavelength of the light source, and usually the sodium D line is employed. Most rotations are measured at 20°C The solvents most commonly employed are water and chloroform

The mutarotation coefficient, $k_1 + k_2$, should be the same for the α- and β-isomers of each sugar. Hudson[34] demonstrated that the alpha and beta isomers of lactose and of some other sugars give identical values for $k_1 + k_2$ and that the mutarotations follow the first-order equation. Table III lists the mutarotation coefficients for several sugars.[35]

TABLE III

Mutarotation Coefficients and Activation Energies for Some Sugars

Sugar	$k_1 + k_2$ (20°C)	Q (cal)	Composition of Equilibrium Solution (%)	
			From rotations	From oxidation studies
α-D-Glucose	0 00632	17,200	α 36 2	37 1
β-D-Glucose	00625	17,200	β 63 8	62 6
α-D-Mannose	0173	16,700	α 68 8	68 9
β-D-Mannose	0178	17,100	β 31 2	31 1
α-D-Xylose	0203	16,800		
α-D-Lyxose	0568	15,300	α 76 0	79 7
β-D-Lyxose	0591	15,700	β 24 0	20 3
α-Lactose H$_2$O	00471	17,300	α 36 8	37 5
β-Lactose	00466	17,600	β 63 2	62 5
α-Maltose H$_2$O	00527	17,500		

The mutarotations of the sugars listed in Table III and those for many other sugars follow the first-order equation. The activation energy averages about 17,000 cal./mole; this value corresponds to an increase in rate of 2.5 times for a 10° rise in temperature. The conformity of the mutarotation data to the first-order equation makes it probable that the main constituents of the equilibrium solution are the α- and β-pyranose modifications. The actual composition may be calculated from the optical rotations of the equilibrium solution when the rotations of the pure α- and β-isomers are known. Data of this type are included in Table III. Independent confirmation of the composition of the equilibrium solutions is provided by studies

[34] C. S. Hudson, *Z physik Chem*, **44**, 487 (1903)

[35] H. S. Isbell and W W Pigman, *J Research Natl Bur. Standards*, **18**, 141 (1937).

of the rates of bromine oxidation of the sugars, the results of which are also found in Table III.

A number of important sugars exhibit mutarotations which do not follow the first-order equation. (See Figures 2 and 3.) A striking case[15] is presented by the pentose ribose; the specific rotation of freshly dissolved L-ribose
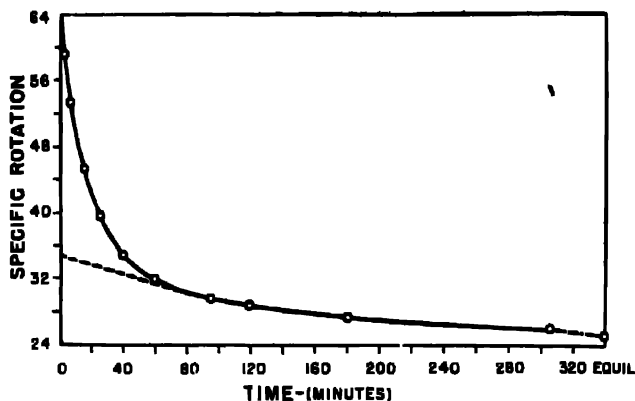
Fig. 2. Mutarotation of α D-talose in water at 0°C.
(Reprinted from *J. Research Natl. Bur. Standards*, *18*, 164 (1937))
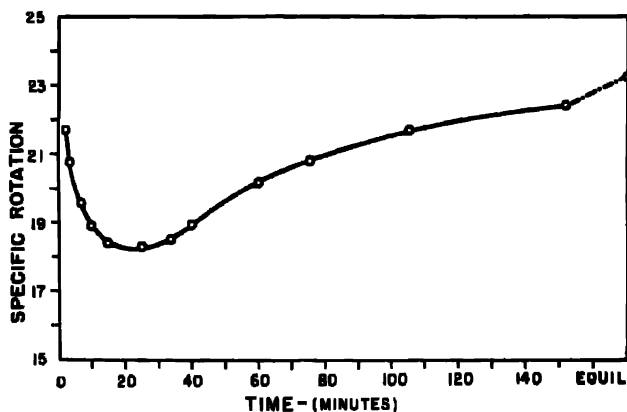
Fig. 3. Mutarotation of L-ribose in water at 0°C
(Reprinted from *J. Research Natl. Bur. Standards*, *18*, 164 (1937))

decreases from an initial value of +23.4 to a minimum of +18.2 and then rises to a constant value of +23.2. Some other sugars such as α- and β-galactose, α- and β-talose and α- and β-arabinose exhibit similar but less striking deviations from the first-order equation. In Fig. 4, log ($r_t - r_∞$) vs time is plotted for α-D-glucose and α-D-talose. Although the curve for α-D-glucose is linear and follows the first-order equation, that for α-D-talose deviates greatly from a straight line during the initial period. This deviation

is an indication of the lack of the conformity of the talose mutarotation with the first-order equation.

In general, the mutarotations which cannot be expressed by the first-order equation conform to equations derived on the assumption of three components in the equilibrium mixture. The equilibrium involved may be:

$$\alpha \leftrightarrows \mu \leftrightarrows \beta \tag{V}$$
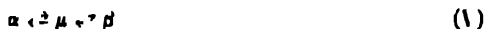
Equations[45][46][47] fitting this condition were derived by Riiber and Minsaas and by Smith and Lowry. The Smith and Lowry type of equation is represented by equation VI.

$$[\alpha] = A \times 10^{-m_1 t} + B \times 10^{-m_2 t} + C \tag{VI}$$
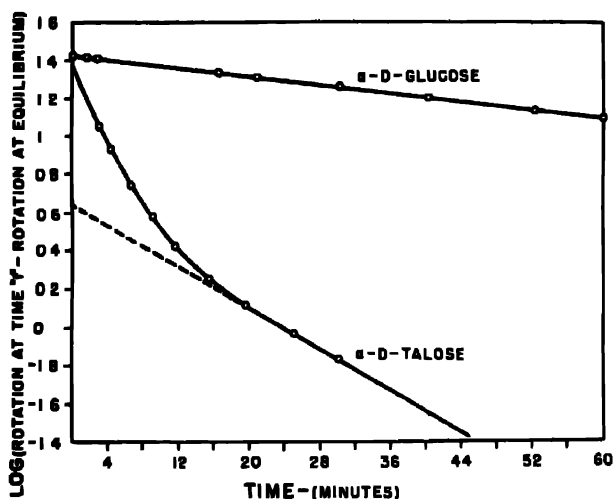
FIG. 4 "Simple" and "complex" mutarotations
(Reprinted from "Polarimetry, Saccharimetry and the Sugars"
by F. J. Bates and Associates.)

In this equation, C is the equilibrium rotation, A is the total change in optical rotation due to the slowly mutarotating component, and B is $(r_0 - r_\infty) - A$. Methods for applying these equations are described elsewhere.[45] The constants m_1 and m_2 are functions of the velocity constants for the various reactions represented in equation V.

Changes in other properties such as the solution volume, the refractive index and the heat content have been shown by Riiber and his associates[46] to parallel the changes in rotations.

Mutarotations which cannot be expressed by the first-order equation but

[46] C. N. Riiber and J. Minsaas, Ber., 59, 2266 (1926)
[47] G. F. Smith and T. M. Lowry. J. Chem. Soc., 666 (1928)

which are expressed by equation VI must represent the establishment of equilibria in which three or more components are present in appreciable quantities. Hence, equilibrated solutions of sugars such as galactose, arabinose, talose and, particularly, ribose must have appreciable quantities of isomers other than the pyranose modifications. The ease of conversion of galactopyranose to furanose and free-aldehyde modifications is shown by the formation of appreciable quantities of such isomers in the products of the acetylation (see p. 152).

The mutarotation reactions which follow equation VI may be considered to consist of two simultaneous or consecutive reactions one of which is slow and the other of which is rapid. The values of m_1 (which represents the reaction constant for the slowest reaction) are about the same as those for $k_1 + k_2$ for glucose, and the activation energies also have closely the same value as for glucose." It is probable then that the slower reactions are α,β conversions between pyranose isomers. The reactions represented by m_2 are 5 to 10 times more rapid, and the activation energy is much smaller (about 13,200 cal./mole as compared with 16,900 for the normal mutarotations). For the rapid mutarotation reactions of galactose, talose and ribose, the magnitude of the reaction constant, the small activation energy and the influence of pH on the rate of mutarotation are similar to those for the mutarotation of the furanose modification of fructose. Since the mutarotation of fructose probably represents mainly a pyranose-furanose change,[38] the fast mutarotations of the other sugars also may represent pyranose-furanose interconversions.

It is usually considered that the interconversion of the alpha and beta isomers and of pyranose and furanose forms takes place through the intermediate formation of the *aldehydo* or *keto* forms of the sugars.

$$\alpha\text{-Glucopyranose} \longleftarrow \quad \longrightarrow \beta\text{-Glucopyranose}$$
$$\downarrow \quad \downarrow$$
Glyconic aldehyde
(or hydrated form)
$$\uparrow \quad \uparrow$$
$$\alpha\text{ Glucofuranose} \longleftarrow \quad \longrightarrow \beta\text{ Glucofuranose}$$
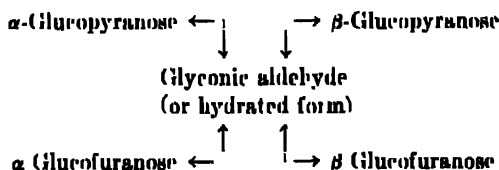
There is no direct proof for the existence of the open-chain forms. However, small quantities of the acetylated open-chain forms are obtained along with the ring forms when some sugars are acetylated (see under Acetyl sugars). Sugar solutions contain isomers which are reducible at the dropping mercury electrode of the polarograph.[39] The amounts of the reducible form present in 0.25 M solutions of several aldoses at pH 7.0 and 25°C. are

[38] H. S. Isbell and W. W. Pigman, *J Research Natl. Bur Standards*, **20**, 773 (1938)
[39] S. M. Cantor and Q. P. Peniston, *J. Am. Chem Soc.*, **62**, 2113 (1940)

shown in Table IV. As may be seen from the table, the amount of the reducible form in glucose solution is very small (0.024 mole per cent). Solutions of other sugars, particularly ribose, contain fairly large amounts The quantity of reducible material increases rapidly as the pH becomes greater. The data obtained by Cantor and Peniston are said to agree with those reported by Lippich[40] for the amount of material in solution which reacts "instantaneously" with hydrocyanic acid. The nature of the reducible form has not been established but most probably it is the aldehyde form in a free or in a hydrated condition. For the sugars listed in Table IV, a correlation exists between the mutarotation velocity and the quantity of the reducible modification in the solution. For this reason, this modification may be the intermediate of the mutarotation reaction. It is also of interest

TABLE IV

Quantity of Reducible Form Present in Solutions of Several Sugars

Sugar	Reducible forms (mole per cent of total sugar)
Glucose	0 024
Mannose	0.064
Galactose	0.092
Allose	(1 38)
Xylose	0.17
Arabinose	0 28
Lyxose	0 40
Ribose	8 5 (0.1 *M*)

that the sugars with the largest quantities of material which are reduced by the dropping mercury electrode are those that exhibit complex mutarotations.

The mutarotation reactions are catalyzed by both hydrogen and hydroxyl ions. The rate of mutarotation of glucose and galactose is at a minimum between the pH limits 3.0 to 7.0. At pH values greater than 7.0 and less than 3.0, the velocity increases rapidly. The curve for mutarotation velocity vs. pH is represented by an inverted catenary. The influence of hydrogen and hydroxyl ions on the rate was found by Hudson to be expressible by equations of the type:

$$k_1 + k_2 = A + B[H^+] + C[OH^-] \tag{I}$$

where A, B and C are constants. For glucose at 20°C. the equation is[38]:

$$k_1 + k_2 = 0.0060 + 0.18[H^+] + 16,000[OH^-] \tag{II}$$

[40] F. Lippich, *Biochem Z.*, **248**, 280 (1932)

According to equation II, glucose mutarotates most slowly at pH 4.61. Acids and alkalies influence the mutarotation of levulose and some other sugars much more markedly than glucose although the minimum for levulose occurs near that for glucose. As may be seen from equations I and II, at pH 4.6 the portion of the catalysis which is due to the water (term A) is much greater than that caused by the hydrogen and hydroxyl ions. In turn, the hydroxyl ions are much more effective catalysts than the hydrogen ions (compare values for B and C). Since water may dissociate according to the equation

$$H_2O \rightarrow H^+ + OH^-$$

it functions as an efficient catalyst. Lowry and Faulkner[41] provide evidence that mutarotations are catalyzed only by solvents which have amphiprotic properties. Thus, mutarotation proceeds slowly in pure pyridine (basic properties) and in cresol (acid properties), but in a mixture of one part pyridine and two parts cresol, the reaction proceeds 20 times as rapidly as in aqueous solution. For aqueous methanol and ethanol solutions, the rate of mutarotation decreases rapidly as the alcohol concentration increases. Even in the anhydrous alcohols and dioxane a very slow mutarotation takes place.[41]

According to combustion data[43] for the crystalline sugars, the complete conversion of α- to β-glucose is accompanied by a heat absorption of 1500 cal./mole and a free energy change of 500 cal./mole.

B. In the Presence of Acids. The mildest type of reaction of the sugars induced by acids is the interconversion between α- and β-isomers or between ring isomers. This type of change has been discussed above under the general subject of mutarotation. Dilute acids at room temperatures have little or no additional action on the sugars, but hot concentrated acids produce profound changes.

The action of acids is that of dehydration. The dehydration may take place by the formation of anhydro rings or of double bonds. The configuration of altrose favors anhydro formation, and the 1,6-anhydroaltropyranose is formed by a brief treatment of the sugar with boiling dilute acids (see under Anhydro sugars). Stronger acids produce furfural, 5-methylfurfural, and 5-hydroxymethylfurfural or levulinic acid from pentoses, 6-desoxy-hexoses and hexoses, respectively. The formation of these materials, in particular furfural from the pentoses, proceeds so well that the reaction is

[41] T. M. Lowry and I. J. Faulkner, *J. Chem. Soc.*, *127*, 2883 (1925); T. M. Lowry, *Z. physik. Chem.*, *130*, 125 (1927)

[42] See H. H. Rowley and W. N. Hubbard, *J. Am. Chem. Soc.*, *64*, 1010 (1942)

[43] H. M. Huffman and S. W. Fox, *J. Am. Chem. Soc.*, *60*, 1400 (1938)

used for their estimation.[44,45] It has been suggested[46] that the reaction of a pentose to give furfural proceeds through the following stages:

$$
\begin{array}{ccccccc}
\text{HCO} & & \text{HCO} & & \text{HCO} & & \text{HCO} \\
| & & | & & | & & | \\
\text{HCOH} & & \text{COH} & & \text{C} \text{---} & & \text{C} \\
| & & \| & & | \quad | & & \| \\
\text{HCOH} & \xrightarrow{\text{Hot acid}} & \text{CH} & \rightarrow & \text{CH} & \rightarrow & \text{CH} \\
| & & | & & | \quad \text{O} & & | \quad \text{O}\\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} & & \text{CH} \\
| & & | & & | \quad | & & \| \\
\text{H}_2\text{COH} & & \text{H}_2\text{COH} & & \text{H}_2\text{C} \quad \text{-} & & \text{HC} - \\
\text{Pentose} & & & & & & \text{Furfural}
\end{array}
$$
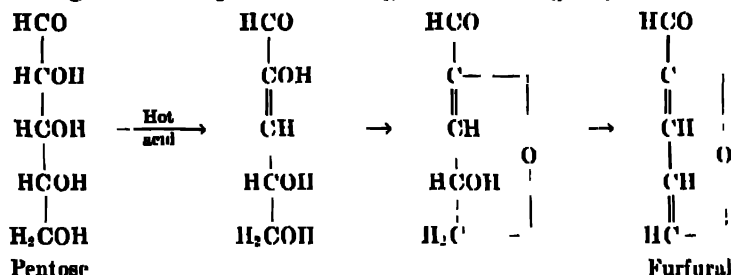
The corresponding derivative produced from the hexoses is 5-hydroxy-methylfurfural which, however, is easily transformed into levulinic acid.[47a]

$$
\begin{array}{ccccc}
\text{HCO} & & \text{HCO} & & \text{COOH} \\
| & & | & & | \\
\text{HCOH} & & \text{C -} & & \text{CH}_2 \\
| & & \| \quad | & & | \\
\text{HCOH} & & \text{CH} & & \text{CH} \\
| & \xrightarrow{-\text{Acids}} & | \quad \text{O} & \text{- - \rightarrow} & | \\
\text{HCOH} & & \text{CH} & & \text{C=O} \\
| & & \| \quad | & & | \\
\text{HCOH} & & \text{C -} & & \text{CH} \\
| & & | & & + \\
\text{H}_2\text{COH} & & \text{H}_2\text{COH} & & \text{HCOOH} \\
\text{Hexose} & & \text{5-Hydroxymethyl} & & \text{Levulinic} \\
& & \text{furfural} & & \text{acid}
\end{array}
$$
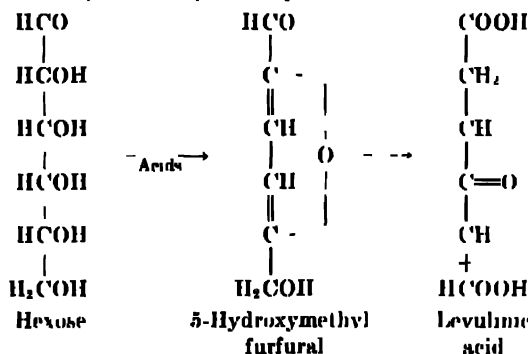
According to Pummerer and Gump,[47b] levulinic acid is formed as a result of the following series of reactions:

$$
\begin{array}{cccccccc}
\text{CHO} & & \text{CHO} & & \text{HCOOH} & & & \text{COOH} \\
| & & | & & + & & & | \\
\text{C ---} & & \text{CO} & & \text{CHO} & & & \text{CH}_2 \\
\| \quad | & \xrightarrow[\text{HCl}]{\text{HOH}} & | & \xrightarrow{\text{HOH}} & | & \xrightarrow{\substack{\text{(intermolecular} \\ \text{oxidation-reduction)}}} & & | \\
\text{HC} \quad | & & \text{CH} & & \text{CH} & & & \text{CH}_2 \\
| \quad \text{O} & & | & & | & & & | \\
\text{HC} \quad | & & \text{CH}_2 & & \text{CH} & & & \text{CH}_2 \\
\| \quad | & & | & & | & & & | \\
\text{C ---} & & \text{CO} & & \text{CO} & & & \text{CO} \\
| & & | & & | & & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & & \text{CH}_3
\end{array}
$$
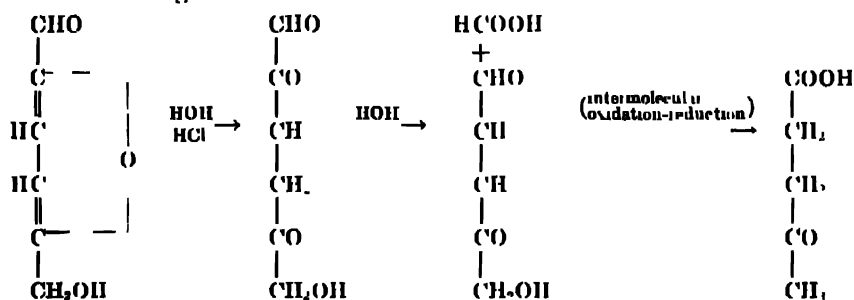
[44] W. E Stone and B. Tollens, *Ann.*, **249**, 227 (1888).

[45] C. A. Browne and F. W Zerban, "Sugar Analysis," p 904; John Wiley, New York (1941).

[46] C. D. Hurd and L. L. Isenhour, *J Am. Chem. Soc.*, **54**, 322 (1932)

[47a] For details of the preparation of levulinic acid see W W Moyer, U S Patent 2,270,328 (Jan. 20, 1942).

Yields of 54 per cent of crystalline hydroxymethylfurfural are reported when a sucrose solution is heated at 145°C in the presence of oxalic acid See: W. N Haworth and W G M Jones, *J Chem. Soc.*, 667 (1944).

Evidence for this mechanism is given by the isolation under similar conditions of the acetal derivative of the intermediate 5-carbon keto aldehyde, and by the high yield of levulinic acid produced from it by the action of acids. Also, yields as high as 80% of levulinic acid have been obtained from 5-hydroxymethylfurfural. It is interesting that two moles of 5-hydroxymethylfurfural readily etherify through the hydroxyl groups when distilled in a partial vacuum (13 mm.).[47a]
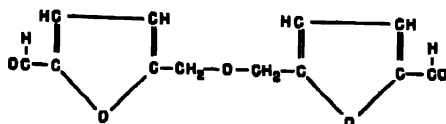
Considerable quantities of "humins" of an unknown nature are formed in the reaction probably as a result of the condensation of the furfural derivatives.

Acids also catalyze the condensation of two sugar molecules to form disaccharides and products (oligosaccharides) of a greater degree of polymerization. In the presence of alcohols, the condensation takes place with formation of the glycosides of the alcohols. A true equilibrium is attained and condensation is favored by a high concentration of the reactants. Condensation appears to take place preferentially between the primary hydroxyl group of one molecule and the reducing group of another molecule.
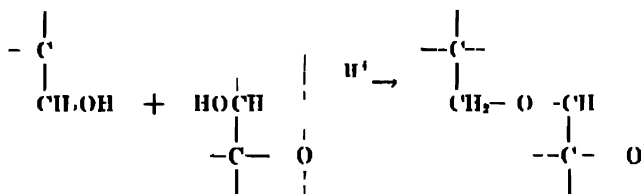
This process is known as "reversion." From glucose, gentiobiose and "isomaltose" ("isogentiobiose") have been prepared (see under Isomaltose).

C. In the Presence of Alkalies. Although the sugars exhibit moderate stability to acids, particularly at room temperature, they are profoundly affected[48] by alkalies even under very mild conditions. Contrary to what might be expected, the sugars exhibit their maximum stability at acid conditions rather than at pH 7. Thus, the optimal pH for the stability of D-

[47a] R. Pummerer and W. Gump, Ber., 56, 999 (1923); R. Pummerer, O. Guyot and L. Birkofer, Ber., 68, 480 (1935).

[47e] J. A. Middendorp, Rec. trav. chim., 38, 1 (1919).

[48] For summary see: W. L. Evans, Chem. Revs., 31, 537 (1942); ibid., 6, 281 (1929).

fructose[49] and for D-glucose[50a] lies between pH 3 and 4. When methylglyoxal production is used as a measure of the stability, the optimal pH is around 1.[50b]

The action of alkalies follows two general courses: Isomerizations mainly at the reducing end of the molecule, and fragmentation into substances that have fewer carbon atoms than the original sugar.

a. ISOMERIZATIONS

The simplest isomerization reaction of the reducing sugars is the Lobry de Bruyn and Alberda van Ekenstein transformation.[51] Thus, when glucose is treated with dilute alkalies at room temperature, the optical rotation decreases. From the products of reaction, glucose, mannose and fructose can be separated. Wolfrom and Lewis[52] found that very few side reactions take place if the glucose is treated with lime water saturated at 35° C. After about five days, the equilibrated mixture had the following composition:

Glucose	63.5 per cent
Fructose	31.0 " "
Mannose	2.5 " "
Other substances (probably saccharinic acids)	3 " "

The treatment of sugars with alkalies has considerable value for preparatory purposes, particularly for obtaining ketoses. Thus, lactose and D-gluco-D-*gulo*-heptose (α-glucoheptose) yield the corresponding ketoses, lactulose and glucoheptulose, respectively (see p. 431).

Early workers were unable to notice any cation effect on the Lobry de Bruyn and Alberda van Ekenstein transformation except in the case of lead hydroxide.[51] Lead hydroxide produced mannose and apparently no fructose from glucose. On the other hand, fructose remained essentially unchanged when treated with lead hydroxide. Kusin[53a] found that calcium hydroxide behaves differently from sodium hydroxide at room temperature. Mannose (but not fructose) could be identified in the products obtained by treating glucose with calcium hydroxide for 24 hours at room temperature, whereas fructose but practically no mannose was found in the product obtained by the action of sodium hydroxide under similar conditions.

For the interpretation of the mechanism of the Lobry de Bruyn-Alberda van Ekenstein transformation, the formation of an intermediate enediol usually is postulated. In the formation of the double bond, the asymmetry

[49] J. A. Mathews and R. F. Jackson, *Bur. Standards J. Research*, **11**, 619 (1933).

[50a] W. Kröner and H. Kothe, *Ind. Eng. Chem.*, **31**, 248 (1939).

[50b] C. Enders, *Biochem. Z.*, **312**, 349 (1942).

[51] C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **14**, 203 (1895); **15**, 92 (1896); **16**, 257, 262, 274, 282 (1897); **18**, 147 (1899).

[52] M. L. Wolfrom and W. L. Lewis, *J. Am. Chem. Soc.*, **50**, 837 (1928).

[53a] A. Kusin, *Ber.*, **69**, 1041 (1936).

of carbon atom 2 is destroyed, and the two epimeric aldoses and the corresponding ketose will be in equilibrium.

$$
\begin{array}{c}
\text{H}_2\text{COH} \\
| \\
\text{C}=\text{O} \qquad \text{Ketose} \\
| \\
-\text{C}- \\
| \\
\updownarrow
\end{array}
$$

$$
\begin{array}{ccc}
\text{HCO} & \text{HCOH} & \text{HCO} \\
| & \| & | \\
\text{HOCH} & \text{COH} & \text{HCOH} \\
| & | & | \\
-\text{C}- & -\text{C}- & -\text{C}- \\
| & | & | \\
\text{Aldose} & \text{Enediol} & \text{Aldose}
\end{array}
$$
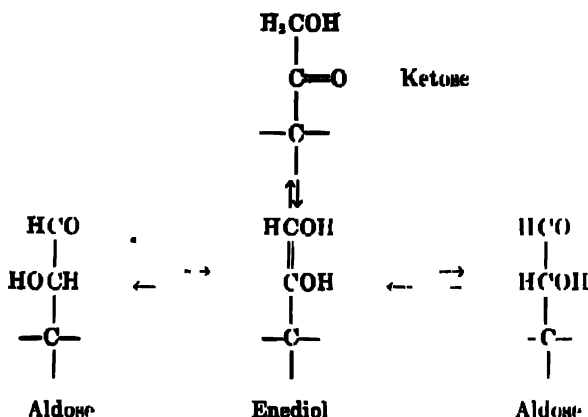
Evidence for the presence of an enediol structure in alkaline solutions of sugars is provided by the ability of the solutions to take up large quantities of iodine, to decolorize solutions of dichloroindophenol and to be oxidized with cleavage between carbons 1 and 2 of the sugar molecule.

As would be expected from this mechanism, 2,3,4,6-tetramethylglucose gives only 2,3,4,6-tetramethylmannose when treated with alkali (saturated lime solutions) The same equilibrium point is reached from tetramethylmannose In this instance, ketose formation is precluded because

$$
\begin{array}{ccc}
\text{HCO} & \text{HCOH} & \text{HCO} \\
| & \| & | \\
\text{HCOCH}_3 & \text{COCH}_3 & \text{CHOCH} \\
| & | & | \\
-\text{C}- & -\text{C}- & \text{C} \\
| & | & |
\end{array}
$$

of the absence of an ionizable hydrogen atom on carbon 2 of the enol form Evidence for the formation of an intermediate enol is provided by the observation that one atom of deuterium is taken up per mole of tetramethylglucose when the isomerization is carried out in heavy water.[38]

Kusin[61a] explains the differences in the actions of calcium and sodium hydroxides by the postulation of a ring structure in the enol formed by a dehydrating action of calcium hydroxide. Such action may involve the intermediate formation of the calcium saccharates of the sugars Since glucose (I) has the ring connected to carbon atom 1 and fructose (IV) to carbon atom 2, the enols (II and III) are not identical and should react

[38b] H. Fredenhagen and K F Bonhoeffer, Z. physik. Chem , A 181, 392 (1938)

differently. On the other hand, the enediol formed by sodium hydroxide does not have a ring structure.

HCOH   HC   HCOH   H₂COH
HCOH   Ca(OH)₂→   COH   C⸺   ←Ca(OH)₂   HOC⸺
    O   O   O   O
-C⸺   -C⸺   -C⸺   -C⸺

(I)   (II)   (III)   (IV)
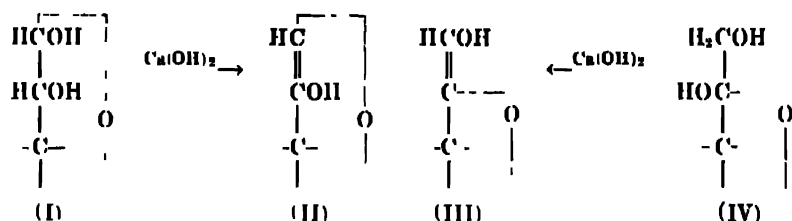
However, the observed differences may arise from the divalent nature of the calcium ion which would enable it to react with contiguous acidic hydroxyls; in contrast, the monovalent cations could react only with a single hydroxyl.

By a continuation of the enolization process, the enediol grouping may move along the carbon chain and additional isomerizations are possible.

HCOH   H₂COH   H₂COH   H₂COH
COH OH→   COH   ⟶   HOC⸺   +   HOC
     ←
HCOH   COH   HCOH   HOCH
                                  O   O
C   -C⸺   -C⸺   C⸺

                        H₂COH   H₂COH
                        HCOH   +   HOCH
                        HOC⸺   HOC⸺
                            O   O
                        -C⸺   -C⸺
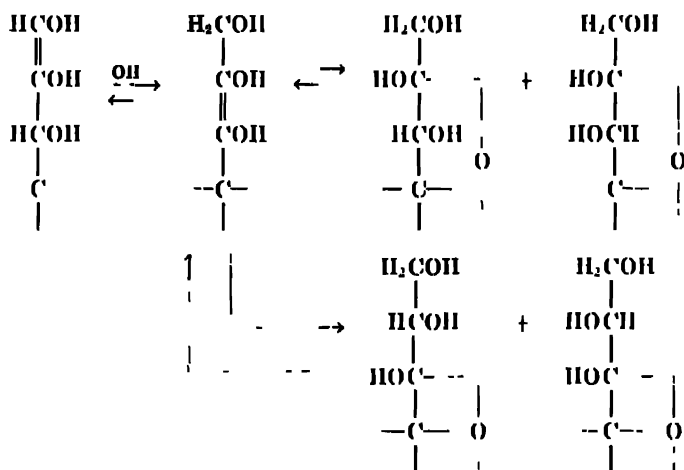
Epiremization of the 2-ketose or the formation of ketoses that have the carbonyl group at carbon 3 may take place. These reactions explain the formation of sorbose from galactose and of allulose from glucose. The unfermentable material remaining after the action of yeasts on a mixture obtained by the treatment of fructose or glucose with dilute alkali has been called "glutose" and a similar product from galactose, "galtose." Lobry de Bruyn and Alberda van Ekenstein[54] considered glutose to be a 3-ketohexose formed by enolization of fructose between carbon atoms 2 and 3. "Glutose" has been characterized as its osazone with a melting point of

[54] C. A. Lobry de Bruyn and W. Alberda Ekenstein, *Rec. trav. chim.*, *16*, 274 (1897); *18*, 72 (1899).

163°C'. An osazone of similar properties can be obtained from the unfermentable reducing fraction of cane sirups and molasses.

Sattler and Zerban[54d] identified the osazone as a mixture of the phenylosazones of glucose and methylglyoxal. The unfermentable fraction ("glutose") contains a mixture of 1,2-anhydrofructopyranose, a difructopyranose anhydride, melanoidins and possibly other substances. Its reducing power is ascribed to 1,2-anhydrofructopyranose which also yields glucosazone under the conditions of osazone formation.

The 3,4 and 1,5-enediols also may be formed. The formation of such enediols would permit of the isomerization of glucose to all of the possible aldohexoses and aldoketoses. Under mild conditions, the enolization probably does not proceed past the 2,3-stage. Such 2,3 enolization is necessary to explain the isolation of allitol by Wolfrom, Lew and Goepp[31] from glucose reduced electrolytically at amalgamated lead cathodes, in mild alkaline solution.

More extensive rearrangement of the sugars in alkaline solution leads to the formation of a group of acids (saccharinic acids) and the corresponding lactones (saccharins). After Peligot (1839) and other workers had isolated acidic materials from among the products of the action of alkalies on glucose, Scheibler and Kiliani[55,56] identified one of the products, saccharinic acid, as an isomer of D-glucose with the empirical formula $C_6H_{12}O_6$. The saccharinic acid loses a molecule of water to form saccharin. The formulas of these substances resulting from the researches of Scheibler and Kiliani are shown below.

COOH
|
CH₂COH

CHOH
|
CHOH
|
CH₂OH

Saccharinic
acid

CO
|
CH₂COH   O
|
CHOH |
|
CH ·
|
CH₂OH
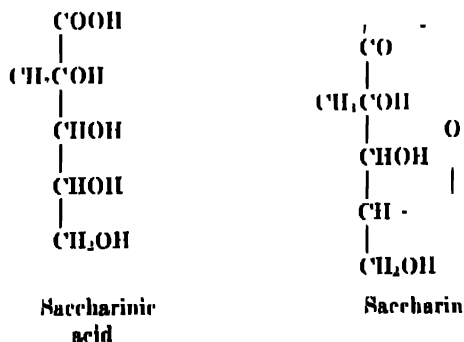
Saccharin

The branched-chain structure of saccharinic acid is demonstrated by the reduction of the acid by hydrogen iodide (and phosphorus) to 2-methyl-

[54d] L. Sattler and F. W. Zerban, Ind. Eng Chem., 37, 1133 (1945).

[31] M. L. Wolfrom, B. W. Lew and R. M. Goepp, Jr., J. Am. Chem. Soc., 68, 1443 (1946).

[55] C Scheibler, Ber., 13, 2212 (1880).

[56] H. Kiliani, Ber., 15, 701, 2953 (1882)

pentanoic acid:

$$\begin{array}{c} CH_3 \\ | \\ CH_3-CH_2-CH_2-CH-COOH \end{array}$$
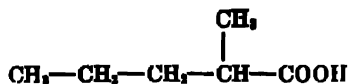
Nitric acid oxidizes the saccharinic acid to a dibasic acid which by reduction with hydrogen iodide is converted to 2-methylglutaric acid:

$$CH_2OH \quad CHOH-CHOH-\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-COOH \quad \xrightarrow{HNO_3}$$

$$COOH-CHOH-CHOH-\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-COOH \quad \xrightarrow{\underset{P}{HI}}$$

$$COOH-CH_2-\overset{\overset{\displaystyle CH_3}{|}}{CH}-CH_2\quad COOH$$
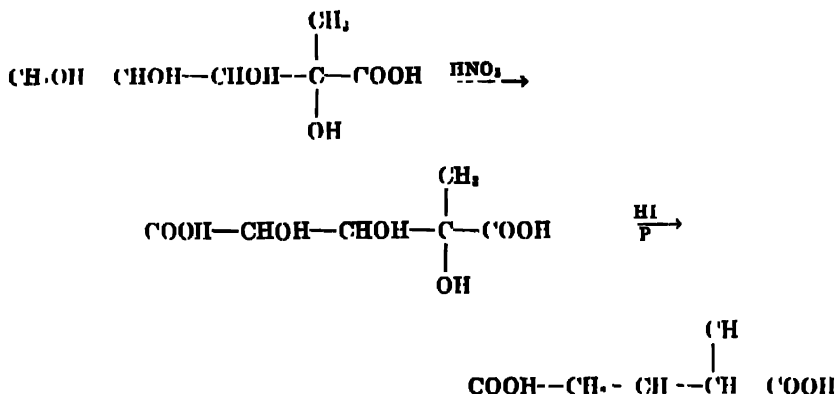
The presence of the new carboxyl group in the terminal position rather than in the 2-position proves that the primary alcohol group is located at the terminal carbon atom and the methyl group is at carbon atom 2.

Cuisinier found among the products of the action of lime on maltose and lactose a substance with the formula $C_6H_{12}O_6$ which was termed isosaccharinic acid. Kiliani prepared the same material from galactose. The corresponding lactone was termed isosaccharin. As a result of the work of Kiliani,[67] the isosaccharinic acids may be formulated as:

$$CH_2OH-CHOH-CH_2-\overset{\overset{\displaystyle CH_2OH}{|}}{COH}-COOH$$

Only two D and two L stereoisomers of such a structure are possible, for only the penultimate ("D,L") carbon atom and carbon atom 2 are asymmetric. The isomers in the D-series are distinguished as α-isosaccharinic acid and β-isosaccharinic acid according to the configuration of carbon atom 2.

Still a third type of product was isolated by Kiliani[68] from the products of the action of alkalies on galactose and lactose. This material, isomeric with saccharinic acid, was termed metasaccharinic acid and is a 3-desoxy-

[67] H. Kiliani, *Ber.*, *18*, 631, 2517 (1885).
[68] H. Kiliani and H. Naegeli, *Ber.*, *35*, 3530 (1902).

aldohexonic acid:

$$CH_2OH - CHOH—CHOH—CH_2—CHOH -COOH$$
Metasaccharinic acid

The mechanism of the formation of the saccharinic acids and their derivatives remains unclear. Nef has suggested that it proceeds from the diketone produced by removal of a molecule of water at carbon atoms 3 and 4 of the ketose. A benzilic acid rearrangement of the diketone would produce the isosaccharinic acids. It is also possible that saccharinic acid formation may result from a polymerization of the products of fragmentation which will be discussed below

Isbell[59] has proposed that the formation of the metasaccharinic acids (II) takes place from the 1,2-enediol (I), whereas the saccharinic (IV) and isosaccharinic acids arise from the 2,3-enediols (III) The formation of these acids may take place as represented below:

Formation of Metasaccharinic Acids (II)
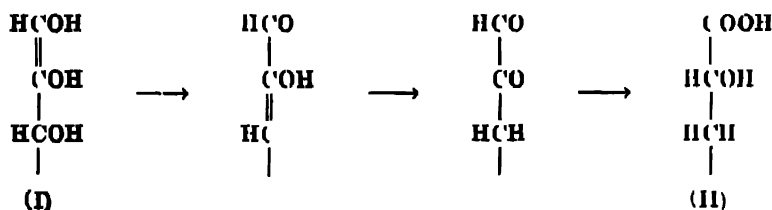
Internal Cannizzaro
Reaction

Formation of Saccharinic Acids (IV)
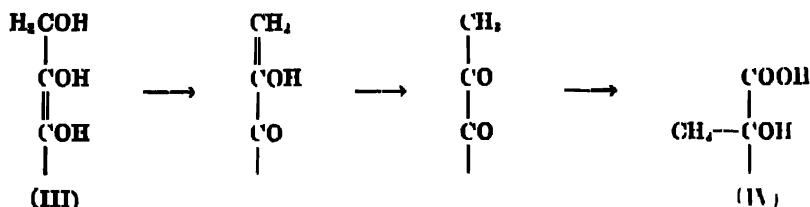
b. FRAGMENTATION

In addition to isomerizations, which proceed mainly through the enediol forms of the reducing sugars, cleavage of the carbon chains also occurs under more drastic conditions. The nature of the products has been elucidated through a long series of researches carried out by Nef.[60a]

Cleavage of the carbon chain may take place to give: (1) formaldehyde

[59] H. S Isbell, J Research Natl. Bur. Standards, 32, 45 (1944)
[60a] J. U Nef, Ann , 357, 294 (1907); 376, 1 (1910), 403, 204 (1913).

and aldopentoses, (2) glycolaldehyde and aldotetroses, or (3) dihydroxy-
acetone and glyceraldehyde. In turn each of these products may isomer-
ize through the corresponding enediols to the corresponding ketoses and
by the saccharinic acid type of rearrangement to the various saccharinic
acids. Nef described the still more complicated mixture obtained in the
presence of oxidizing agents as a "furchtbares Gemisch," but it would seem
that the term could be applied aptly to the mixture obtained without the
additional complication of oxidation reactions. One of the principal prod-
ucts which may be obtained by cleavage of the carbon chain is *dl*-lactic
acid. From one mole of glucose, treated at 25°C. with benzyltrimethylam-
monium hydroxide, Evans[18] reports the production of 1.2 moles of lactic
acid (60 per cent of theory). The lactic acid may be considered as the
saccharinic acid related to glyceraldehyde. Some other short-chain products
that have been identified are: dihydroxybutyric acid, glyceraldehyde, di-
hydroxyacetone, methylglyoxal (pyruvaldehyde), formaldehyde, acetol,
diacetyl, formic and acetic acids, and reductone (the enol of hydroxymalonic
aldehyde).

The cleavage of the carbon chain probably takes place through a reversed
aldol condensation. The aldol condensation of glyceraldehyde and dihy-
droxyacetone to form ketohexoses has been demonstrated (see p. 113).

$$
\begin{array}{ccc}
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
| & & | \\
\text{CO} & & \text{CO} \\
| & & | \\
\text{CH}_2\text{OH} & \text{OH} & \text{CHOH} \\
| & \rightleftarrows & | \\
\text{CHO} & & \text{CHOH} \\
| & & | \\
\text{CHOH} & & \text{CHOH} \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH}
\end{array}
$$
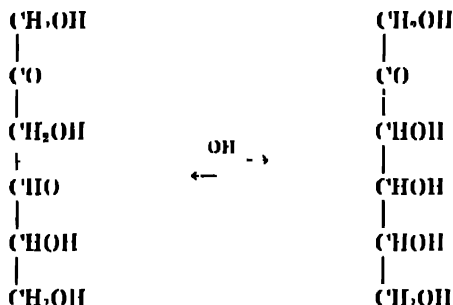
There is little doubt but that the cleavage of the carbon chain of sugars
takes place in this manner under nonoxidizing conditions, and glycer-
aldehyde, dihydroxyacetone and methylglyoxal have been isolated. Similar
cleavages also could take place to give glycolaldehyde and aldotetroses;
the cleavage between carbons 1 and 2 would give formaldehyde and an
aldopentose. Schmidt,[40b] however, explains the cleavage of the carbon chain
by assuming that in the 1,2-enediol the bond in the 3,4-position is weak-
ened by the presence of the double bond, and that it is the enediol which
undergoes the cleavage of the 3,4-bond.

40b O. Schmidt, *Chem. Revs.*, 17, 137 (1933)

As is evident from the above discussion the action of alkalies on the sugars is a complicated and still little understood process in spite of the extensive and excellent work by Nef, Evans and many other workers. Very dilute alkalies catalyze the α, β and presumably the furanose-pyranose conversions. In greater amounts, they bring about isomerization between the epimeric aldoses and the corresponding ketoses, probably through the formation of a 1,2-enediol. Higher concentrations of alkalies bring about conversions between all of the various sugars of the same chain length, probably as a result of the formation of 2,3 and 3,4-enediols. Cleavage of the carbon chain also takes place with formation of the 2, 3, 4 and 5-carbon sugars. Rearrangements occur in which saccharinic acids are formed from the original sugars and from their isomerization and cleavage products. Finally, as shown by Nef, polymerization takes place with the formation of resins and "polysaccharides" of unknown composition.

D. Behavior of the Sugars with Short Carbon Chains. The foregoing discussions of the sugars in solution mainly was devoted to the hexoses. The aldopentoses and the higher carbon sugars may be expected to exhibit similar reactions because they can form pyranose and furanose rings and enediols similar to those for the hexoses. As the number of carbon atoms decreases, pyranose and finally furanose rings become impossible. Thus, the ketopentoses and the aldotetroses can form only furanose rings; the trioses and glycolaldehyde cannot form even a furanose ring. Ring formation appears to take place when possible as is demonstrated by the mutarotation[51] of crystalline D-threose ($[\alpha]_D$ $+29.1 \rightarrow +19.6$) and by the normal molecular weight of erythrose in solution.[52] The lower sugars with two and three carbon atoms form dimers easily; for some of the substances both the monomeric and the dimeric forms have been isolated.[53] The dimers of glycolaldehyde and of glyceraldehyde have been formulated[54] as the products of an extramolecular acetal formation analogous to the formation of pyranose and furanose rings by the higher sugars:

$$
\begin{array}{ccc}
\text{H}_2\text{C}\!-\!\text{CHO} & & \text{H}_2\text{C}\!-\!\text{CH(OH)} \\
| \quad\;\; | & \rightleftarrows \;\rightarrow & |\quad\;\; | \\
\text{OH\;OH} & & \text{O}\quad\text{O} \\
| & & |\quad\; | \\
\text{OHC}\!-\!\text{CH}_2 & & \text{(HO)HC}\!-\!\text{CH}_2
\end{array}
$$
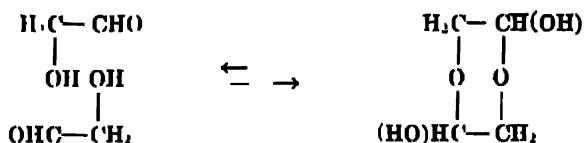
[51] W. Freudenberg, *Ber.*, **65**, 168 (1932).

[52] V. Deulofeu, *J. Chem Soc.*, 2973 (1932).

[53] For example, see results for dihydroxyacetone as reported by: H. O. L. Fischer and H. Mildbrand, *Ber*, **57**, 707 (1924).

[54] M. Bergmann and A. Miekeley, *Ber.*, **62**, 2297 (1929); **64**, 802 (1931); R. K. Summerbell and L. K. Rochen, *J Am Chem. Soc.*, **63**, 3241 (1941); E Baer and H. O. L. Fischer, *J. Biol. Chem.*, **150**, 213 (1943); A. Wohl and C. Neuberg, *Ber.*, **35**, 3095 (1900).

In conformity with the conversion of glucose to mannose and fructose, glyceraldehyde is converted partially to dihydroxyacetone in the presence of pyridine or dilute alkalies.[55]

$$
\begin{array}{ccc}
\text{CHO} & & \text{CH}_2\text{OH} \\
| & & | \\
\text{CHOH} & \underset{\longleftarrow}{\overset{\text{OH}^-}{\longrightarrow}} & \text{CO} \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH}
\end{array}
$$
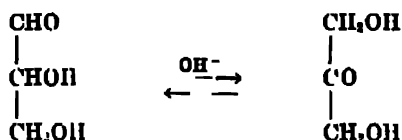
At 25°C. to 50°C., potassium hydroxide (0.2 to 6 N) converts glyceraldehyde to formic, acetic and lactic acids.[56]

6. Optical Superposition, the Isorotation Rules and the Influence of Structure on Optical Rotation

In optically active compounds that have more than one asymmetric center, the rotation of each compound might be considered as the sum of the partial rotations of the asymmetric centers. Thus, for the isomeric compounds:

$$
\begin{array}{cccc}
\text{R} & \text{R} & \text{R} & \text{R} \\
| & | & | & | \\
\text{HCOH}\ (+a) & \text{HCOH}\ (+a) & \text{HCOH}\ (+a) & \text{HOCH}\ (-a) \\
| & | & | & | \\
\text{HCOH}\ (+b) & \text{HCOH}\ (+b) & \text{HOCH}\ (-b) & \text{HCOH}\ (+b) \\
| & | & | & | \\
\text{HCOH}\ (+c) & \text{HOCH}\ (-c) & \text{HCOH}\ (+c) & \text{HCOH}\ (+c) \\
| & | & | & | \\
\text{R} & \text{R}' & \text{R}' & \text{R}' \\
\text{(I)} & \text{(II)} & \text{(III)} & \text{(IV)}
\end{array}
$$
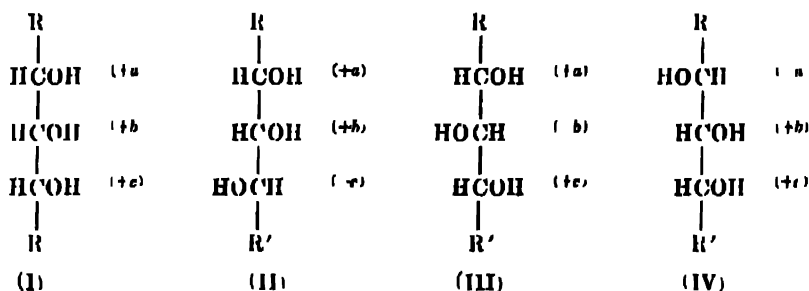
the partial rotations contributed by the individual asymmetric carbon atoms might be represented as $\pm a$, b, and c. If in all of the above stereoisomers, the rotatory contribution of each asymmetric center remains the same and differs only in sign according to its configuration, the sum of the rotations of compounds II, III and IV should be equal to that of the compound I. Thus,

For compound I,   the rotation is $+a+b+c$
For compound II, the rotation is $+a+b-c$
For compound III, the rotation is $+a-b+c$
For compound IV, the rotation is $-a+b+c$

Sum (II+III+IV) is $(+a+b+c)$

[55] H. O. L Fischer, C. Taube and E Baer, *Ber.*, **60**, 480 (1927).
[56] W L. Evans and H B. Hass, *J. Am. Chem. Soc.*, **48**, 2703 (1926).

The hypothesis of the additive nature of the rotatory contributions of the individual asymmetric centers of stereoisomers in making up the total rotation of each isomer was formulated by van't Hoff and has been known as the "principle of optical superposition." In its full generalization as applied to all substances, the hypothesis of optical superposition is definitely unsound and thus it is not a "principle"; nevertheless, it has been shown by Hudson[17] that the hypothesis holds in first approximation for a large number of carbohydrates, and the approximation is sufficiently close to permit valuable inferences concerning structure and configuration to be drawn from comparisons of the rotations of carbohydrates through the application of his Isorotation Rules.

According to these rules, the rotation of a glycoside or other sugar derivative may be considered to be composed of two parts: A, the partial rotation of the anomeric carbon atom, and B, the rotatory contribution of the other active centers. According to the configuration of the active centers, A and B may be positive or negative.

Alkyl α-D glucoside

$[M]_\alpha = +A +B$
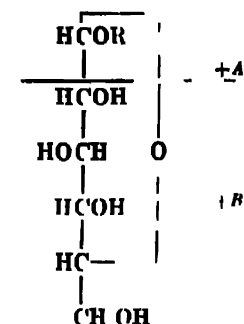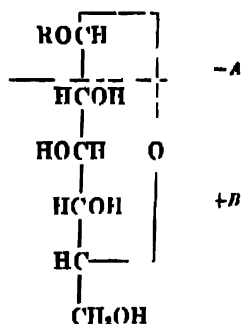
Alkyl β-D-glucoside

$[M]_\beta = -A +B$

The application of the optical superposition principle permits of the calculation of the partial rotations A and B Thus, $M_\alpha - M_\beta = A + B + A - B = 2A$ and $M_\alpha + M_\beta = A + B - A + B = 2B$. Hence, the partial rotations may be obtained by adding the molecular rotations of anomers to give $2B$ and by subtracting the molecular rotation of the beta isomer from that of the alpha isomer to give $2A$. The partial rotations are one-half of each of these sums and differences As a result of the measurement of the rotation of many alpha-beta pairs in the sugar series, Hudson was able to formulate the two Rules of Isorotation:

Rule 1: "The rotation of Carbon 1 in the case of many substances of the

[17] C. S. Hudson, *J Am Chem Soc*, **31**, 66 (1909) See also F. J Bates and Associates, "Polarimetry, Saccharimetry and the Sugars," Circular C 440 of the Natl Bur. of Standards, p. 411 (1942).

sugar group is affected in only a minor degree by changes in the structure of the remainder of the molecule."

Rule 2: "Changes in the structure of carbon 1 in the case of many substances of the sugar group affect in only a minor degree the rotation of the remainder of the molecule."

According to the first rule, changes in the structure of a sugar or glycoside molecule at carbon atoms 2, 3, 4, 5 and 6 should have little influence on the partial rotation (A) of carbon atom 1. In Table V, the effects of substitutions in the pyranose ring of glucosides on the rotatory contribution of the anomeric carbon atom (A) are indicated. As a first approximation, the substitution of methyl groups at carbon atoms 2 and 3 and of large glucosyl groups at carbon atoms 4 and 6 appear to affect the rotation of carbon

TABLE V

Test of Rule 1   2A Values for Substituted Glucosides

Glucopyranoside	Molecular rotation		2A ($M_\alpha - M_\beta$)
	α isomer	β isomer	
Methyl	30,860	− 6,640	37,500
Methyl 2,3 dimethyl	31,690	−8,130	39,800
Methyl 6 β glucosyl- (methyl gentiobiosides)	23,340	− 12,800	36,100
Methyl 4 β glucosyl (methyl cellobiosides)	34,490	6,810	41,300
Ethyl (pyranosides)	31,700	−7,600	39,300
Ethyl (furanosides)	20,400	− 17,900	38,300

atom 1 only to a minor degree. Even a difference in ring structure has but little influence.

Although the first rule does not mention configurational changes, it is of interest to investigate the influence of variations in the configuration of the remaining carbon atoms on the rotatory contribution of carbon 1 For this purpose, the 2A values of a number of glycosides are given in Table VI. It will be noted that the 2A values for the upper four pairs of glycosides agree very well but that the values for the mannosides and rhamnosides differ appreciably from those for the other glycosides. The latter two pairs differ from the others in the configuration of *carbon 2* which is immediately adjacent to carbon atom 1. The observed differences probably are to be ascribed to interaction between the groups attached to carbon 2 and those at carbon 1. As is shown by the 2A values for glucosides, galactosides and gulosides, configurational changes at carbon atoms more distant from carbon 1 than carbon 2 have only a secondary influence on the partial rotation of carbon 1. It would be expected that the unknown idosides, altrosides and talosides (which have the same configuration for carbon

atom 2 as mannose) would have $2A$ values similar to those for the mannosides, whereas the rotational differences for the allosides should be similar to those for the glucosides. The interaction between groups should become less as the temperature is increased. Actually at 80°C., the difference between the $2A$ values for mannose and glucose derivatives is much less[68] than at 20°C.

TABLE VI

$2A$ Values for Glycosides

Methyl glycosides of	$\pm 2A$
L-Arabinose	37,460
D-Galactose	38,220
D-Glucose	37,500
D-Gulose	39,390
D-Mannose	28,930
L-Rhamnose	28,140

TABLE VII

Test of Rule 2—$2B$ Values for Glucosides

Glucoside	$2B (M_\alpha + M_\beta)$
H (glucose)	23,600
Methyl	24,200
Ethyl	24,000
Propyl	22,700
Ethylene glycol	23,500
Allyl	19,700
Cyclohexyl	21,100
Benzyl	21,000
Phenyl	31,400
p-Nitrophenyl	33,700
p-Hydroxyphenyl	31,300

The second Rule of Isorotation requires for each sugar type that the total rotatory contribution (B) of all carbon atoms except that of the anomeric carbon atom (A) be independent of the structure of the groups attached to the latter. Data for testing this rule are given in Table VII by a comparison of the $2B$ values for glucose and the glucosides. For the aliphatic glucosides, there is good agreement between the various $2B$ values. But, as pointed out by several writers, the phenyl glucosides exhibit appreciably larger $2B$ values.[69] The average $2B$ value for the aliphatic glucosides is

[68] W. Kauzmann, *J. Am. Chem. Soc.*, *64*, 1626 (1942)

[69] E. F. and K. F. Armstrong, "The Carbohydrates," p. 41; Longmans, Green &

23,200 ($B = 11,600$) and for the aromatic glucosides is 32,200 ($B = 16,100$). Other sugars exhibit similar differences. These data prove the general validity of the second rule but indicate that the rule should be modified to allow for the differences between the B values for the aromatic and the aliphatic glycosides.

The second Rule of Isorotation has considerable value for the determination of the structure of the sugars. As mentioned elsewhere (p. 46), the structures of the glycosides can be determined by reliable methods, but the corresponding methods for the sugars are less trustworthy. However, if by application of the second rule the sugar is found to have the same B value as a glycoside of known structure, it usually may be assumed that the sugar has the same structure as the glycoside. As an example, the B value for the crystalline forms of glucose may be compared to those for the ethyl glucopyranosides and the ethyl glucofuranosides (Table VIII).

TABLE VIII

2B Values for Glucopyranosides and Glucofuranosides

	$[M]_D$	$2B$
α-D Glucose	20,200	23,600
β-D Glucose	3,400'	
Ethyl α glucofuranoside	20 400	2,500
Ethyl β-glucofuranoside	17,900	
Ethyl α glucopyranoside	31 700	24,100
Ethyl β glucopyranoside	7,600	

The agreement of the $2B$ value for the crystalline forms of glucose with that for the ethyl glucopyranosides provides strong evidence that the known isomers of glucose are pyranose modifications. In the case of glucose, the pyranose structure also is confirmed by other methods (p. 46).

In a similar fashion, application of the Isorotation Rules led to the inference[70] that the biose constituent of the glycoside amygdalin is gentiobiose; this structure was established shortly afterwards by chemical synthesis.

The calculation of the A and B values requires that the rotations of both the alpha and beta isomers be known. However, a direct correlation between the molecular rotations of β-glucosides and the corresponding rotatory contributions (A) of the anomeric carbon atom has been shown. This correlation would be expected, for according to the Isorotation Principle, the molecular rotation of a β-glucoside is represented as $[M]_\beta = -A + B$.

Co , London (1931), W W Pigman and H S Isbell, *J Research Natl Bur. Standards,* **27**, 9 (1941)

[70] C. S Hudson, *J. Am Chem. Soc ,* **46**, 483 (1924).

$[M]_\beta$ should vary directly with A since B is a constant. It is possible then to investigate the effect of the structure of the aglycon group of a glucoside on the partial rotation of the carbon atom 1 by a direct comparison of the molecular rotations of the β-glucosides. The accompanying Tables IX and X list the molecular rotations of many β-glucosides. The values for the molecular rotations that are given in the tables are calculated from the specific rotations by multiplication by the molecular weights $([M]_\beta = [\alpha]_D \times$

TABLE IX

Molecular Rotations of Phenyl β-D-Glucosides

Substituent Group	$[M]_D$		
	When in o position	When in m position	When in p position
None (H)	(−16,190)		
OH	19,330	19,170	− 17,290
OCH	19,260		
NH			17,630
CH₁	18,570	−18,840	18,300
C₂H	18,480		18,590
CH₂ NH	' 18,320	19,540	ᵇ 20,000
CH NH CO CH	10,900	20,190	17,610
CH₂ COOH		−	19,800
CH₃ CO OCH₃			19,010
COOH	18,480	20,540	21,140
CO OCH	− 20,210	23,290	24,550
NO₂	26,500	27,110	31,030
CH CN	− 19,670		21,080
CO CH	19,840		28,120
CHO	17,900		− 26,860

ᵃ Molecular rotations ($[\alpha]_D \times$ mol. wt.) for aqueous solutions at approximately 20°C

ᵇ In aqueous acetic acid solution with acid concentration equivalent to the glucoside concentration

ᶜ One of the values may be in error

M.W.). They represent the influences of variations in the structure of the aglycon group (group R) on the total molecular rotation and probably on A.
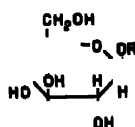
Although the β-glucosides of the primary and secondary alcohols have rotations usually falling in the interval −6,500 to −10,000, those derived

TABLE X
Molecular Rotations of Aliphatic β-D-Glucosides

Aglycon Group	$[M]_D$ in water t = approx. 20°C.	$[\alpha]_D$
Primary Alcohol Series		
CH_3-	-6,640	-34.2
CH_3-CH_2-	-7,640	-36.7
CH_2OH-CH_2-	-6,860	-30 6
$CH_3-CH_2--CH_2-$	-8,600	-38 7
$CH_2OH-CH_2- CH_2$	-8,620	-36 2
$CH_3-(CH_2)_3-$	-8,720	-36 9
$CH_2OH-(CH_2)_3$	-8,830	-35
$CH_3-(CH_2)_4-$	'-8,910	-33 7
$CH_3-(CH_2)_7-$	*-8,860	-30 3
$CH_3-(CH_2)_8-$	*-8,820	-28 k
$CH_3- (CH_2)_9-$	*-8 910	-27 8
$CH_3-(CH_2)_{11}-$	'-8,610	-24.7
$CH_3--(CH_2)_{15}--$	'-8,900	-22.0
Iso Series		
$(CH-)_2CH -$	- 8 070	-36 3
$(CH_3)_2CH- CH_2-$	-9 430	-39 9
$(CH_3)_2CH- CH_2 CH$	-9 110	-36 4
Tertiary Alcohol Series		
$(CH_3)_3C$	1 190	19 0
$(CH_3)_2C(C_2H_5)$	1 480	-17 9
$CH_3C(C_2H_5)_2-$	-4,190	-17 0
$(C_2H_5)_3C$	-3 730	ᵇ-13 4
Cyclohexyl and Benzyl Homologous Series		
$C_6H_{11} -$	-10 860	-41 4
$C_6H_{11} -CH_2$	-10,220	-37 0
$C_6H_{11} -(CH_2)_4$	-5,140	27 3
$C_6H_5-(CH_2)$	-8,670	-30 5
$C_6H_5 -CH_2$	11 460	-53 5
C_6H_5-	-18 190	-71
$m-CH_2OH -C_6H_4 -CH_2$	-14,080	-46 9
$p-CH_2OH-C_6H_4- CH_2$	-15,170	-50 5
Substituted Alcohol Series		
$CH_2=CH -CH_2$	-9,290	-12 2
$COOH- CH_2$	-10,500	-44 1
$NH_2-CO-CH_2-$	-10,250	-43 2
$(CH_2OH)_2CH -$	-7,650	-30 1
$CH_2(OCH_3) -CH_2$	-6,840	-28 7
$CH_2(OC_2H_5) CH_2$	-6,460	-25 6
$CH_2(Cl)-CH_2-$	-7,090	-29 2
$CH_2(Br) -CH_2-$	-7,490	-26 1
$CH_2(I)-CH_2-$	-8,450	-25 3
$CH_2(SO_3H) -CH_2$	-9,480	-32.9
$CH_2(SO_2C_2H_5)-CH_2 -$	-7,620	-24.1
$NH_2 -CO-NH-N=CH- CH_2$	-8,880	-31 8
$CH_2OH-CH_2-O-CH_2-CH_2-$	-6,010	-22 4

ᵃ Rotations measured in CH_3OH at 25° C.
ᵇ For monohydrate
ᶜ Rotation measured in C_2H_5OH at 24°C.
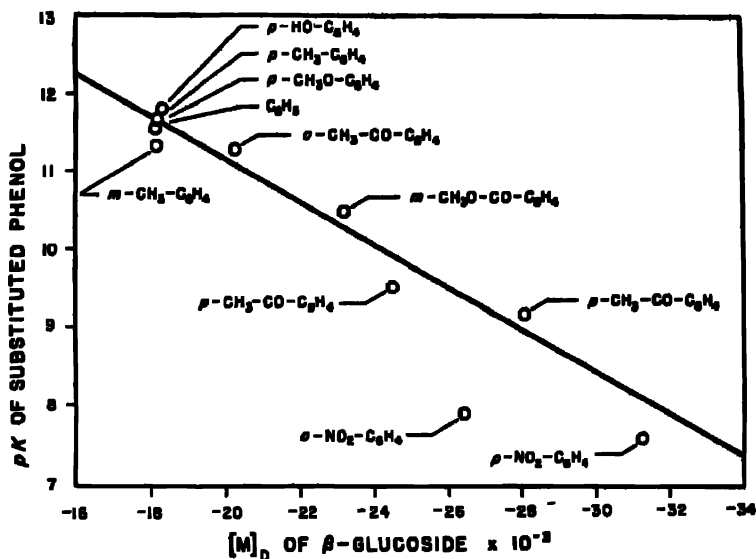
Fic. 5. Relationship between the pK values of phenols and the molecular rotations of the corresponding β-glucosides.
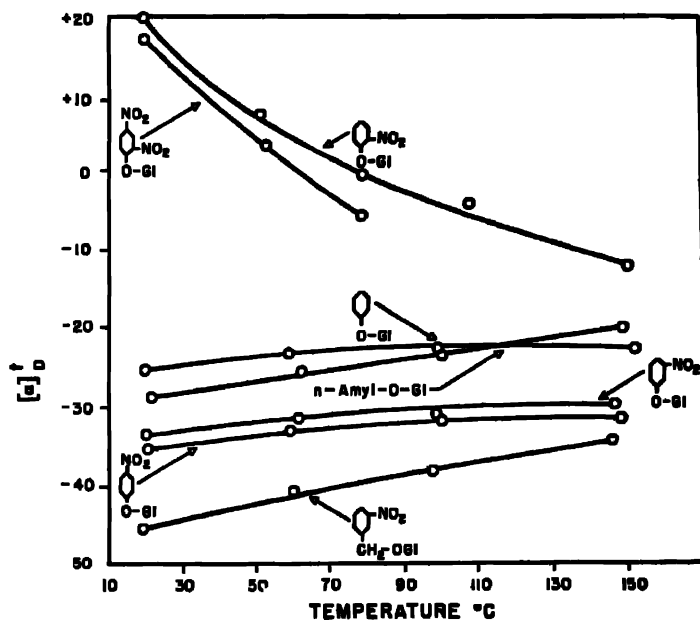
Fig. 6. Influence of temperature on rotation of some β-glucosides.

from phenols exhibit molecular rotations greater than −17,000. The corresponding derivatives of the tertiary alcohols have molecular rotations near −4,000. For the aromatic glucosides, there is an interesting correlation be-

tween the effect of substituent groups present in the phenyl nucleus on the rotations and the influence of the same groups on substitution reactions of benzene derivatives. The "ortho-para directing groups" when substituted in the aromatic nucleus of phenyl β-glucoside have little or no effect on the rotation. However, "meta directing groups" in positions meta and para to the glucosidic connection cause the rotation of the glucoside to become appreciably more negative than for phenyl β-glucoside. Thus, the value of $-31,000$ for p-nitrophenyl β-glucoside compares to that of $-18,200$ for the phenyl β-glucoside. Diortho-substituted derivatives have anomolously low molecular rotations which are near those of the tertiary-alkyl β-glucosides $(-4,000$ to $-5,000)$.

As shown in Fig. 5, the influence of substituents in the aromatic nucleus of phenyl β-glucoside parallels the effect of the same groups on the acidity of the corresponding substituted phenols.

Many of the ortho-substituted phenyl β-glucoside tetraacetates have anomalous positive rotations. Thus, the o-nitrophenyl β-glucoside tetraacetate has a molecular rotation of $+21,100$ ($[\alpha]_D = 15$) as compared to the negative values $-17,400$ and $-19,200$ for the meta and para isomers. However, as shown in Fig. 6, the positively rotating derivatives have a very large temperature coefficient and their rotations become negative at higher temperatures, although the rotations of the m- and p-isomers are affected only to a minor degree by an increase of temperature. This and other evidence makes it probable that the positive rotation of certain of the o-substituted phenyl β-glucoside tetraacetates is due to a bonding of the group in the ortho position with an acetyl group in the sugar portion of the molecule.[71]

[71] W. W. Pigman, *J. Research Natl. Bur. Standards*, 33, 120 (1944).

OCCURRENCE, PROPERTIES, SYNTHESIS AND ANALYSIS OF THE MONOSACCHARIDES

1. Naturally Occurring Monosaccharides

A. Introduction. Many sugars are found free or combined in naturally occurring materials. These sugars are of particular importance because of the interest in their biological function and in their present or potential industrial application. To the sugar chemist, these sugars are of value in providing along with the sugar alcohols and uronic acids starting materials for the preparation of the synthetic sugars. Of course, it is to be expected that some of the sugars now classified as synthetic sugars will in the future be found in natural products.

D-Glucose, free or combined, undoubtedly is the most widely distributed of the sugars. Other aldohexoses found in natural products are D-mannose and D- and L-galactose. Two ketohexoses, D-fructose and L-sorbose, are also encountered, although L-sorbose may be a secondary product produced by the action of bacteria on its precursor, D-sorbitol. The origin of D-psicose (D-allulose) found[1] in the unfermentable fraction of cane molasses remains to be explained. Of the pentoses, D-xylose, D-ribose, D- and L-arabinose and possibly L-lyxose[2] are of biological origin. A ketopentose (L-xylulose) has been reported present in the urine of patients with pentosuria. The lower homologs of the sugar series are represented by the trioses, glyceraldehyde and dihydroxyacetone, which occur as their phosphate derivatives in the intermediate stages of yeast fermentation as well as in many other important biological processes. D-Mannoheptulose and sedoheptulose are the only known naturally occurring sugars with carbon chains longer than six atoms, although two seven-carbon alcohols (perseitol and volemitol) have been isolated from plant products.

Many desoxysugars, formally derived from ordinary sugars by the replacement of a hydroxyl group by a hydrogen atom, are of biological origin. The methyloses, with a terminal methyl rather than a primary alcohol group, are the most common desoxysugars. All of the aldohexoses mentioned above are represented in the D- or L-form by naturally occurring methyloses which are 6-desoxy-D-glucose, 6-desoxy-D- and L-galactose (D- and L-fucose), and 6-desoxy-L-mannose (L-rhamnose). The occurrence of L-rhamnose rather than its enantiomorph is perplexing since the

[1] F. W. Zerban and L. Sattler, *Ind. Eng. Chem.*, **34**, 1180 (1942); L. Sattler, U. S. Patent 2,377,853, June 5, 1945.

[2] J. M. Gulland and G. R. Barker. *J Chem. Soc.*, **625** (1943).

corresponding natural aldohexose (D-mannose) belongs to the D- rather
than the L-series. Desoxysugars other than the methyloses are found as
constituents of nucleic acids (*Chapter IX*) and of the cardiac glycosides
(*Chapter XI*).

It is of interest that only sugars of the galactose type are to be found in
both the D- and L-forms. Thus, D- and L-galactose, D- and L-fucose and
D- and L-arabinose are constituents of natural products. The occurrence
of both the D- and L-forms may be a result of the symmetry of the galactose
molecule, for reduction of the hemiacetal group and oxidation of the pri-
mary alcohol group would result in the conversion of D-galactose to L-
galactose.

$$
\begin{array}{ccc}
HC{=}O & \xrightarrow{\;+H_2\;} & H_2COH \\
| & & | \\
HCOH & & HCOH \\
| & & | \\
HOCH & & HOCH \\
| & & | \\
HOCH & & HOCH \\
| & & | \\
HCOH & & HCOH \\
| & & | \\
H_2COH & \xrightarrow{\;-H_2\;} & HC{=}O \\
\text{D-Galactose} & & \text{L-Galactose}
\end{array}
$$
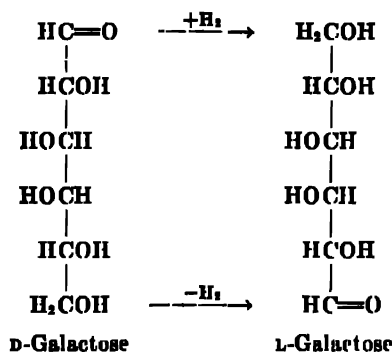
Two sugars with branched carbon-chains (apiose and hamamelose)
have been reported (see p. 470). The presence of still other monosaccharides
has been recorded, but additional investigation is necessary before the
identification can be accepted. L-Glucose, the enantiomorph of the uni-
versally occurring D-glucose, has been reported[3, 4] to be among the hy-
drolysis products of the glycoside capsularin from jute leaf and to be in the
leaves of *Grindelia* species.

The natural sugars may exist free, or combined as components of larger
molecules such as oligosaccharides, polysaccharides, glycosides, etc. The
better-defined polysaccharides are usually homopolymers of monoses;
cellulose, starch and glycogen are glucose polymers; inulin gives D-fructose,
and pectins yield mainly D-galacturonic acid on hydrolysis. Other poly-
saccharides (gums and hemicelluloses) are heteropolymers of simple
sugars, uronic acids and amino sugars. Many of the rarer sugars appear
as constituents of glycosides or nucleic acids.

**B. Properties, Identification, Origin and Preparation of Naturally Occuring
Monosaccharides.** Many particulars of interest in connection with the individual

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[3] F. B. Power and F. Tutin, *Chem. Zentr.*, **77**, II, 1623 (1906).
[4] H. Saha and K. N. Choudhury, *J. Chem. Soc.*, **121**, 1044 (1922).

naturally occurring monoses are given in the following pages. For additional information and details, it is suggested that the following references in particular be consulted:

"Beilsteins Handbuch der organischen Chemie," Vol. 31; J. Springer, Berlin (1938).

F J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars"; Circular C 440 of the National Bureau of Standards; U S. Gov't. Printing Office, Washington, D. C. (1942).

a. HEXOSES

D-Fructose

(HO)H$_2$C — C — C — C — C — CH$_2$OH
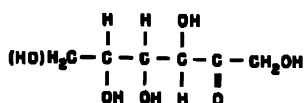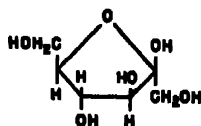
(keto-Fructose)   (β(?)-Fructofuranose)

Synonyms. Levulose, fruit sugar.

Properties. m.p., 102–104°C.; $[\alpha]_D^{20} = -132.2 \rightarrow -92.4$ (H$_2$O; c, 4). Fermentable by yeasts.

Identification. α-Methylphenylosazone, o-, m- and p-nitrophenylhydrazones, (glucose) phenylosazone and numerous ketose color reactions.

Occurrence. D-Fructose is found, usually accompanied by sucrose, in an uncombined form in fruit juices and honey. Apples and tomatoes are said to have particularly large quantities of the sugar. Sucrose consists of D-fructose and D-glucose in glycosidic union. Plants of the family *Compositae* contain large amounts of levulose polysaccharides (inulins) of the type of inulin. It is of interest that many common weeds, *e.g.*, Jerusalem artichoke, burdock, goldenrod and dandelion, as well as dahlias and chicory utilize inulins as reserve polysaccharides. The sugar is a frequent constituent of oligosaccharides, often combined with glucose as a sucrose unit, but it rarely occurs in glycosides other than oligosaccharides.

Preparation.[5] The abundance and wide distribution of D-fructose in natural products, its sweetness and its resistance to crystallization have stimulated considerable experimental work on methods of preparation. Most methods depend on the formation of a difficultly soluble calcium levulate or fructosate in which one mole of the sugar is combined with one of lime. The compound is washed free from impurities, such as other sugars and inorganic salts, and decomposed to D-fructose and insoluble calcium carbonate by carbonation.

The best source of D-fructose for large-scale purposes is probably the

[5] R. F. Jackson, C. G. Silsbee and M. J. Proffitt, *Bur. Standards Sci. Papers, 20*, 587 (1926); T. S. Harding, *Sugar, 25*, 406 (1923).

For a comparison of different sources see: E. S Haber, W. G. Gaessler, and R. M. Hixon, *Iowa State Coll J. Sci., 16*, 201 (1942).

inulins of the Jerusalem artichoke, a native sunflower, or others of the plants mentioned above (*Compositae*). These polysaccharides are extracted from the artichoke tubers with hot water, the extracts are hydrolyzed by acids (or possibly by enzymes) and the D-fructose is precipitated from the solution with lime. For small preparations, sucrose is frequently utilized as the raw material by inverting it with acids or invertase and then separating the fructose and D-glucose. The separation is accomplished by direct crystallization, by removal of the glucose by oxidation to gluconic acid (ketoses are not affected), or by the separation of the calcium fructosate. Conditions are patented for preparing fructose by the action of alkali on D-glucose.[1]

General Discussion. Only one crystalline isomer of the sugar is known and this is probably the pyranose form. In solution, however, as indicated by evidence obtained from mutarotation studies (p. 67), a considerable amount of the furanose modification is present. There is no evidence for a true equilibrium between α- and β-isomers although this condition may be a result of the presence of only a small quantity of the unknown isomer rather than of its complete absence. Upon acetylation, the acetylated acyclic modification is obtained accompanied by the cyclic forms. In natural products the sugar, when combined, is always found as the furanose modification.

Most tests have shown D-fructose to be the sweetest of the sugars, although the actual ratios between the various sugars depend to a considerable extent on the methods and conditions adopted for the comparison. Taking the sweetness of sucrose as 100, that for D-fructose has been reported as varying from 103 to 173. The following table gives the relative sweetness of some sugars and other organic compounds.

TABLE I

Relative Sweetness of Some Organic Compounds[1]

	Relative Sweetness
Cane sugar (sucrose	1
D Fructose	1 0-1 5
D Glucose	0 5-0 6
Lactose	0 27
Maltose	0 60
Sorbitol	0 48
Glycerol	0 48
Invert sugar	0 8-0 9
Saccharin	200-700
Perillaldehyde α-*anti* aldoxime	2000

[1] S M Cantor and K C Hobbs, U S Patent 2,354,664, Aug 1944

[1] C F Walton, "International Critical Tables," 1, 357 (1926)

D-Fructose is not easily prepared because it is very soluble and crystallizes with difficulty, but these properties are advantageous in the preparation of sirups and candies which will not crystallize.

D-Galactose
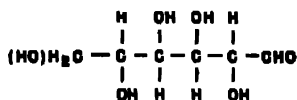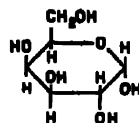
(aldehydo D-Galactose)　　　　(α D Galactopyranose)

Synonyms. Cerebrose, "brain sugar."

Properties. α-Isomer; m.p., 167°C; $[α]_D^{20}$ = 150.7 → 80.2 (H_2O; c, 5).

β-Isomer; $[α]_D^{20}$ = 52.8 → 80.2 (H_2O; c, 4).

Fermentable by lactose yeasts or yeasts especially acclimatized to galactose.

Identification. α-Methylphenylhydrazone, galactose oxime, oxidation to mucic acid.

Occurrence. The sugar is a frequent constituent of oligosaccharides, notably lactose, melibiose and raffinose. Polysaccharides which yield galactose on hydrolysis include agar, gum arabic, mesquite gum, western larch gum and many other plant gums and mucilages. A few glycosides have also been reported to yield galactose on hydrolysis (idaein, myrtillin, the cerebrosides, etc.). Crystalline galactose has been observed on ivy berries.

Galactogen, a galactosan found in the albumin gland of the snail (*Helix pomatia*) and frog spawn are also rich in galactose. The cerebrosides, occurring in considerable amounts in brain and nerve tissue, are galactosides.

Preparation[8]. The most frequently used method requires the hydrolysis of lactose by acids and the direct fractional crystallization of the galactose. A modification[9] of the method involves the removal of the glucose by fermentation with yeasts and the crystallization of the remaining galactose. Water-soluble gum from the western larch is suggested[10] as a source of galactose. A similar gum is extractable from the eastern larch[11] (see under Arabo-galactan).

General Discussion. Two crystalline isomers of the sugar are known. The α-form is the stable form, obtained under most conditions. The β-isomer is prepared by crystallization from cold alcoholic solution[12].

Galactose and glucose differ only in the configuration of carbon 4, and

[8] T. S. Harding, *Sugar*, **25**, 175 (1923); E. P Clark, *Bur. Standards Sci. Papers.*, **17**, 228 (1922).

[9] G. Mougne, *Bull soc. chim. biol*, **4**, 206 (1922)

[10] A. W. Schorger and D. F Smith, *J. Ind. Eng Chem*, **8**, 194 (1916).

[11] L. E. Wise, P. L. Hamer and F C. Peterson *Ind Eng. Chem*, **25**, 184 (1933)

[12] C. S. Hudson and E. Yanovsky, *J Am Chem Soc.*, **39**, 1021 (1917).

this difference results in a greater tendency for galactose to give furanose derivatives. As a result, the mutarotation of the galactose isomers does not follow the first-order equation, and considerable quantities of furanose isomers are formed when the sugar is directly acetylated (p. 152).

Galactose is one of the few sugars other than D-glucose which are found distributed to any great extent in the animal kingdom. In combination with glucose as the disaccharide lactose, it is an important constituent of the milk of mammals. The *in vitro* conversion of glucose to lactose through the enzymic action of mammary tissue extracts has been demonstrated. The reaction does not seem to involve a direct condensation of glucose and galactose but more probable proceeds through a degradation and resynthesis (see under Lactose). The kidney threshold of galactose is very low but despite this the sugar is metabolizable. When galactose is injected intravenously into normal dogs, it is removed from the blood within two hours, but only 10 to 30 per cent appears in the urine[13]. The manner of its utilization is uncertain, however. The effect of the ingestion of galactose on glycogen formation by fasted rabbits has been investigated by Bell,[14] who although unable to detect any galactose in the glycogen formed, reports that the glycogen molecule consists of repeating units of 18 glucose residues rather than the 12 usually found for these animals on normal diets. However, from such livers a small amount of D-galactose 1-phosphate has been isolated which is unaffected by enzymes of muscle, liver and yeast extracts but which is rapidly fermented by galactose-adapted yeasts.[15] There is little difference in the galactose tolerance of diabetic and normal men. In rabbits, insulin does not accelerate the disappearance of galactose from the blood.[16] However, in many clinical conditions impaired galactose clearance from the blood has been demonstrated.[17a] D-Galactose, like D-xylose, differs from glucose, fructose, mannose and arabinose in that when fed to young rats in large quantities (35 per cent of the diet) it produces cataracts.[17b] When still greater amounts are ingested (55 per cent of galactose in diet), chickens develop violent spasms and die after a few days.[17c]

Cerebrosides.[18] A group of poorly defined substances known as cerebro-

[13] J. L Bollman, F. C Mann and M. H. Power, *Am. J. Physiol.*, *111*, 483 (1935)

[14] D. J. Bell, *Biochem. J.*, *30*, 1612 (1936)

[15] H. W. Kosterlitz, *Nature*, *144*, 635 (1939); S. P. Colowick, *J. Biol. Chem.*, *124*, 557 (1938).

[16] J. H. Roe and A S. Schwartzman, *J. Biol. Chem.*, *96*, 717 (1932).

[17a] A. M. Bassett, T. L. Althausen and G C. Coltrin, *Am. J Digestive Diseases*, *8*, 432 (1941).

[17b] See: H S. Mitchell and G. M. Cook, *Proc. Soc. Exptl. Biol. Med.*, *45*, 85 (1940); A. M. Yudkin and H. A. Geer, *Arch. Ophthalmol.*, *23*, 28 (1940).

[17c] H. Dam, *Proc. Soc Exptl Biol. Med.*, *55*, 57 (1944).

[18] General reference: I. H. Page, "Chemistry of the Brain", p. 140; Charles C. Thomas; Springfield, Illinois (1937). E. Klenk and K. Schuwirth, *Ann. Rev. Biochem.*, *6*, 123 (1937).

sides or galactolipins are found in brain and nervous tissue and in other tissues and organs. The investigations of Thudichum, Thierfelder, Levene, Rosenheim and Klenk have established that the cerebrosides yield on hydrolysis a nitrogenous base (sphingosine), a fatty acid and a sugar According to Klenk,[19] a special group of cerebrosides, found only in central nervous tissue, may be distinguished as gangliosides.

Alkaline hydrolysis of the cerebrosides yields fatty acids and a glycosyl-sphingosine:

$$CH_3-(CH_2)_{12}-CH=CH-CH-CH-O-C_6H_{11}O$$
$$\underset{OH}{|} \quad \underset{CH_2-NH_2}{|}$$
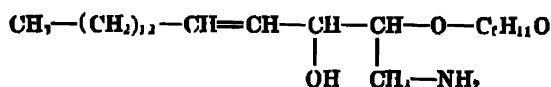
The fatty acid constituent of kerasin is lignoceric acid,

$$CH_3-(CH_2)_{22}-COOH,$$

of phrenosin is

$$CH_3-(CH_2)_{21}-CHOH-COOH;$$

and of nervonic acid is

$$CH_3-(CH_2)_7-CH=CH-(CH_2)_{12}-CH_2-COOH$$
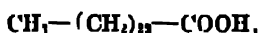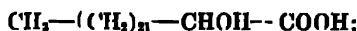
These cerebrosides apparently consist of the glycosides of an α-hydroxy-amine (sphingosine) in combination with different fatty acids which are combined with the base through an amide (peptide-like) linkage. Nervonic acid, for example, is given the following formula:

$$CH_3-(CH_2)_7-CH=CH-(CH_2)_{13}-C=O$$
$$\underset{OH}{|} \qquad \underset{NH}{|}$$
$$CH_3-(CH_2)_{12}-CH=CH-CH-CH-CH_2$$
$$\underset{O-C_6H_{11}O, \quad (galactosyl)}{|}$$
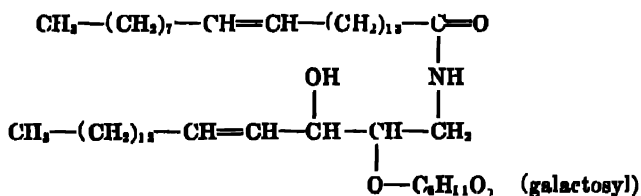
Nervonic acid

Although the principal sugar component of cerebrosides is D-galactose, a cerebroside resembling kerasin and separated from the spleen of a patient suffering from Gaucher's disease has glucose rather than galactose as the sugar constituent.[20] The cerebrosides are believed to be glycosides since some are hydrolyzed by almond emulsin[21, 22] (α- or β-galactosidase-?).

[19] E Klenk, Z. physiol. Chem., 273, 76 (1942).

[20] N. Halliday, H. J Deuel, Jr., L. J. Tragerman and W. E. Ward, J. Biol Chem , 132, 171 (1940). See also: J. Brückner, Z physiol. Chem., 275, 73 (1942); J. Polonovski, Bull. soc. chim. biol., 25, 44 (1943).

[21] B. Helferich, H. Appel and R. Gootz, Z. physiol Chem., 215, 277 (1933).

[22] J. Pryde and R. W. Humphreys, Biochem. J., 20, 825 (1926).

Since methylation and hydrolysis of oxbrain cerebroside produces[22] tetra-methylgalactopyranose, the sugar residues in the cerebrosides have a pyranoside structure.

L-Galactose; D,L-Galactose
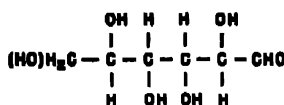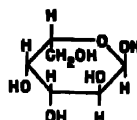
(aldehydo-l-Galactose)　　　(α-l-Galactopyranose)

Properties. See more accurately determined properties of the D-isomer

    D,L-Isomer: m.p. 143 144°, 163°C., optically inactive

    L-Galactose is not fermented by yeasts.

Occurrence. Several polysaccharides including chagual gum, agar-agar, and flaxseed mucilage produce L-galactose on hydrolysis, and since D-galactose is usually present, the D,L-galactose is obtained. Galactogen from snails also gives D- and L-galactose on hydrolysis.[23]

Preparation. The synthetic methods are the most convenient although the preparation from flaxseed mucilage and agar has been described.[24] The separation of L-galactose from natural or synthetic D,L-mixtures is accomplished by the fermentation of the D-galactose by galactose-adapted yeasts or by resolution of the hydrazones formed from optically active (d-amyl)-phenylhydrazine.[25]

The reduction of the readily available D-galacturonic acid to L-galactonic acid and finally to L-galactose may be recommended for the preparation of this sugar. More details are given later in this chapter (p. 129). It would be desirable that the alkaline rearrangement of L-sorbose[26] be given additional investigation as a means for obtaining L-galactose

D-Glucose
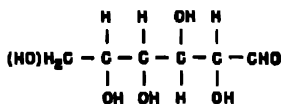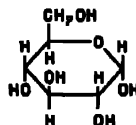
(aldehydo D-Glucose)　　　(α-D-Glucopyranose)

[22] D J Bell and E Baldwin, *Nature*, *146*, 559 (1940)

[24] E Anderson, *J Biol. Chem*, *100*, 249 (1933); C Araki, *J. Chem Soc Japan*, *59*, 424 (1938).

[25] C Neuberg and M. Federer, *Ber*, *38*, 872 (1905).

[26] C A Lobry de Bruyn and W Alberda van Ekenstein, *Rec trav chim.*, *16*, 245 (1897), *19*, 10 (1900).

Synonyms. Dextrose, blood sugar, grape sugar, corn sugar.

Properties. α-Isomer; m.p., 146°C.; $[\alpha]_D^{20} = +112.2 \rightarrow +52.7$ (H_2O; c, 4)

α-Isomer (hydrate); m.p., 83°C.; $[\alpha]_D^{20} = +102.0 \rightarrow +47.9$ (H_2O; c, 4)

β-Isomer; m.p., 148–150°C.; $[\alpha]_D^{20} = +18.7 \rightarrow +52.7$ (H_2O; c, 4)

Fermentable by yeasts.

Identification. Phenylosazone, p-nitrophenylhydrazone, oxidation to saccharic acid and separation as silver salt, formation of double salts with sodium chloride.

Occurrence. This sugar, in a free or combined form, is not only the most common of the sugars but also is probably the most abundant organic compound. It occurs free in fruits, plant juices, honey, blood, lymph, cerebrospinal fluid and urine and is a major component of many oligosaccharides (notably of sucrose), polysaccharides (particularly cellulose, starch and glycogen) and glycosides.

Preparation.[27] D-Glucose is manufactured on a large scale from starch. Potato starch (Europe) and corn starch (America) are utilized.

Starch, in aqueous suspension, and 0.25 to 0.5 per cent of hydrochloric acid (by weight of starch) are put in a converter. Steam is passed into the converter and a pressure of about 40 pounds/in.^{2} is maintained until a 90 to 91 per cent conversion to glucose has been attained. The acid solution is then passed into tubs and neutralized to a pH of 4.8 with sodium carbonate. Fatty materials originating from the starch are removed by centrifugals, and protein and insoluble carbohydrates subsequently by filtration. The filtrate is decolorized and purified by passing through bone black (animal charcoal) and after evaporation to approximately 30°Bé (ca 55 per cent by weight) is filtered again through bone black. The final filtrate is then evaporated in a vacuum pan. The subsequent treatment depends upon the product desired.

The last stage in the process is the most difficult to carry out on a large scale because the crystallization should take place from aqueous solution (cheapness), the crystals should be homogeneous (at least three forms are possible) and the particular crystals obtained should be easily centrifuged and washed. The conditions under which the various pyranose forms of D-glucose are stable are illustrated in the following phase diagram[28] of the system (D-glucose)-water (Fig. 1).

Below 50°C., α-D-glucose·H_2O is the stable crystalline phase but above 50°C. the anhydrous form is obtained. At still higher temperatures, the β-D-glucose forms the solid phase. Although at any temperature it is usu-

[27] W. B. Nowkirk, *Ind Eng. Chem.*, *16*, 1173 (1924); *28*, 760 (1036); *31*, 18 (1039)

[28] W B Newkirk, *Ind Eng. Chem.*, *28*, 764 (1936)

ally possible to obtain any form by the addition of the proper seed crystals, this is usually not desirable since the introduction of seed crystals of the more stable modification will result in a change to the latter if equilibrium conditions are attained. In the commercial process these conditions are met for the hydrate by cooling the liquid at a concentration of about 40°Bé. (about 77 per cent by weight) to a temperature of about 120°F. (50°C.), and after seeding heavily with the hydrate allowing it to crystallize while
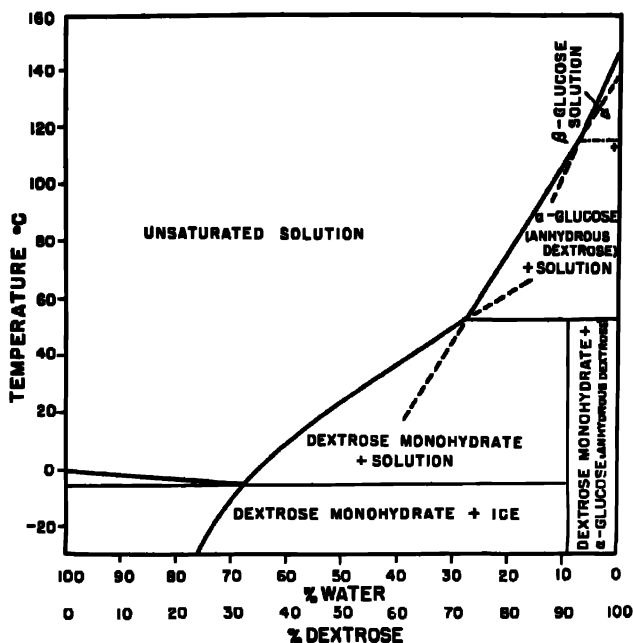
Fig. 1. Phase diagram of the system dextrose-water.
(Based on data from the National Bureau of Standards and the Geophysical Laboratory.
(W. B Newkirk, *Ind Eng. Chem* , *28*, 764, 1036)

the mass is stirred and slowly cooled. The crystals are then separated by centrifugation and passed through driers.

For the preparation of the anhydrous material, the crystals are developed at higher temperatures in the vacuum pan while the evaporation is taking place. This is done by first evaporating about 15 to 20 per cent of the total batch to a thick sirup (90 per cent dry substance) and allowing crystals to form spontaneously. The remainder of the batch is then used to dilute the seed formed and the evaporation is continued. When the crystals have developed to the desired point, the mass is passed into a centrifuge and the mother liquors removed. During this final stage and during the washing,

the proper temperatures are maintained to prevent spontaneous formation of the hydrate. The crystals are finally dried by filtered, warm air.

The β-D-glucose has proved of some technical interest because of its greater initial solubility. It has been prepared[29] by dissolution in hot pyridine and crystallization at 0°C. The accompanying molecule of pyridine is removed at 105°C. The β-isomer is also prepared[30] by crystallization from hot acetic acid and recrystallization from water and alcohol at lower temperatures. At temperatures greater than about 115°C., the β-D-glucose is the stable form in contact with a saturated aqueous solution (see Fig. 1). Because of the high solubility at these temperatures very concentrated solutions must be used. It is possible to work at somewhat lower temperatures (100°C.) if seed of the α-isomer is excluded. The β-D-glucose may be prepared by seeding a concentrated glucose solution at 100°C. with β-glucose and then evaporating it at this temperature to a solid mass.[31]

In industrial circles, the term "glucose" is used to describe a partially hydrolyzed starch product that consists of dextrins, oligosaccharides, maltose and dextrose (D-glucose). The material is also designated as C.S.U. (corn sirup, unmixed). The commercial material is made by autoclaving aqueous starch suspensions with acids. It has a reducing power usually in the range 40 to 45 per cent of the same weight of dextrose; the concentration of solid material lies in the range 78 to 85 per cent.

"Hydrol" is the mother liquors remaining from the preparation of dextrose and corresponding to the "molasses" of cane sugar refining. It contains dextrose, disaccharides and oligosaccharides. Gentiobiose can be isolated from the material (see under Gentiobiose). This disaccharide and some other oligosaccharides probably are reversion products produced by the condensation of dextrose in the presence of the acids during the original hydrolysis.

<div align="center">

D-Mannose

</div>
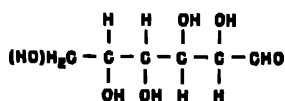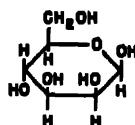
(aldehydo-D-Mannose)   (β-D-Mannopyranose)

Synonyms. Seminose.

Properties. α-Isomer; m.p., 133°C.; $[\alpha]_D^{20} = +29.3 \rightarrow +14.2$ (H$_2$O; c, 4).

[29] R. Behrend, *Ann*, *377*, 220 (1910); A. W. Mangam and S. F. Acree, *J. Am. Chem. Soc.*, *39*, 965 (1917).

[30] C. S. Hudson and J. K. Dale, *J. Am. Chem. Soc*, *39*, 323 (1917).

[31] R. L. Whistler and B. F. Buchanan, *J. Biol. Chem.*, *125*, 557 (1938); C. Tanret, *Bull. soc. chim.* [3] *13*, 733 (1895).

β-Isomer; m.p., 132°C.; $[\alpha]_D^{20}$ = $-17.0 \rightarrow +14.2$ (H_2O; c, 4). Fermentable by yeasts.

Identification. Phenylhydrazone, anhydro hydrazone tetraacetate, reduction to mannitol.

Occurrence. Authentic instances of the presence of the free sugar in natural products are lacking but polysaccharides yielding mannose on hydrolysis are frequently encountered. For preparatory purposes, the most important source is the seed of the tagua palm,[32] *Phytelephas macrocarpa*, also known as vegetable ivory. Salep mucilage from tubers of *Orchidaceae*, white spruce hemicellulose and *Phoenix canariensis* are rich enough sources of mannose so that they have been used for the preparation of this sugar. Mannose has also been reported as a constituent of ovomucoid of blood serum globulins and of tubercle bacilli.

Preparation.[33] Shavings obtained as by-products from the preparation of buttons from the ivory nut (*Phytelephas macrocarpa*) are considered the best source. The vegetable ivory shavings are hydrolyzed with acids, and by a fractionation employing alcohols, the mannose formed is separated from other substances and crystallized directly from alcoholic solution or alternatively is converted to the easily crystallizable methyl α-mannoside. The direct crystallization of mannose is a considerable improvement over the earlier methods which separated the sugar as the phenylhydrazone.

General Discussion. Two pyranose isomers of the sugar are known, and either may be obtained from aqueous solution by adding seed crystals of the desired form to a supersaturated solution. The importance of having seed crystals is well illustrated by this sugar. The single isomer known for many years was the β-D-mannose, but in laboratories in which the alpha isomer had been obtained, it became very difficult to obtain the more soluble β-form. The β-D-mannose now can be obtained only by very careful exclusion of the seed of the α-isomer.

Mannose forms an easily crystallizable compound[34] with calcium chloride of the formula $C_6H_{12}O_6 \cdot CaCl_2 \cdot 4H_2O$ which exhibits a complex mutarotation with a maximum and which appears to contain the furanose modification of the sugar.[35]

D-Mannose and the related L-rhamnose are the only known natural sugars with the configuration of carbon 2 different from that of glucose.

[32] R. Reiss, *Ber.*, **22**, 609 (1889).

[33] T. S. Harding, *Sugar*, **25**, 583 (1923); E. P. Clark. *J Biol. Chem.*, **51**, 1 (1922); C. S. Hudson and E. L Jackson, *J. Am. Chem. Soc.*, **56**, 958 (1934); H. S. Isbell, *J. Research Natl. Bur. Standards*, **26**, 47 (1941).

[34] J. K. Dale, *Bur. Standards J. Research*, **3**, 459 (1929)

[35] H. S. Isbell, *J. Am. Chem. Soc.*, **55**, 2166 (1933); H. S. Isbell and W. W Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Since the configuration of this carbon influences many of the reactions and properties of the groups on the neighboring carbon 1, mannose exhibits some difference from glucose, particularly in certain reactions involving groups on carbon 1. The contribution of carbon 1 to the rotation of the entire molecule is less for mannose (and rhamnose) than for glucose. When reacted with alcohols in the presence of silver carbonate, the acetyl-mannosyl halides give orthoesters rather than the mannosides, owing to the *trans* configuration of the 2-acetyl and 1-halogeno groups. As a result it is difficult to prepare normal β-derivatives of mannose, *e.g.*, methyl β-D-mannoside.

D-Mannose is absorbed by rats at only about 12 per cent of the rate of glucose, and even after allowance for this difference in absorption, the glycogen deposition in the liver is much smaller for mannose than for glucose. This sugar is also much less effective than glucose in lowering an existing ketonuria.[36]

L-Sorbose

$$(HO)H_2C - C - C - C - C - CH_2OH$$
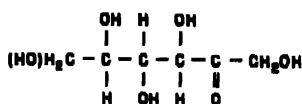
(*keto* L-Sorbose)
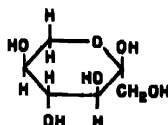
$$\text{(a(?) L-Sorbopyranose)}$$

Synonyms. Sorbinose, also in earlier literature *d*-sorbose.

Properties. m.p., 159–161°C.; $[\alpha]_D^{20} = -43\,7 \rightarrow -43.4$ (H_2O; *c*, 12).

Not fermentable by yeasts.

Identification. Phenylosazone.

Occurrence. Although L-sorbose is found in the fermented juice of mountain ash berries (*Sorbus aucuparia* L.), it has been shown to be a secondary product formed by the oxidation of D-sorbitol by bacteria such as *Acetobacter xylinum* (see p. 236).

Preparation. The biochemical oxidation of sorbitol is the most convenient source of this sugar which, as an intermediate in the commercial synthesis of ascorbic acid, is prepared in large quantities by this method. The early researches by Bertrand[37] showed that sorbitol may be oxidized by sorbose bacteria (*Acetobacter xylinum* Adrian Brown) to L-sorbose. Yields of 50 75 per cent are reported.[38] By carrying out the fermentation with *Aceto-*

[36] H. J Deuel, Jr., F. Hallman, S. Murray and J. Hilliard, *J. Biol. Chem*, *136*, 79 (1938)

[37] G. Bertrand, *Compt rend*, *126*, 762 (1896).

[38] H. Schlubach and J. Vorwerk, *Ber.*, *66*, 1251 (1933).

bacter suboxydans in rotating drums instead of utilizing surface cultures of Bertrand's organism, yields of over 90 per cent are obtained.[39]

b. PENTOSES

L-Arabinose

OH OH H
| | |
(HO)H$_2$C — C — C — C — CHO   ,
| | |
H   H   OH
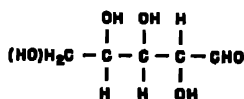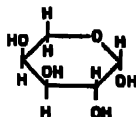
(*aldehydo*-L-Arabinose)   (β-L-Arabinopyranose)

Properties. m.p., 160°C.; $[\alpha]_D^{20} = +190.6 \rightarrow +101.5$ (H$_2$O).
Not fermentable by yeasts.

Identification. Diphenylhydrazone, α-benzoylhydrazone, benzylphenylhydrazone, p-nitrophenylhydrazone, phenylosazone.

Occurrence. The sugar, in a combined form, is very widely distributed in plant products, being found in gums, hemicelluloses, pectic materials and bacterial polysaccharides. Spent beet pulp is considered an excellent source. Several glycosides yield the sugar on hydrolysis.

Preparation.[40] Mesquite gum, from a plant (*Prosopis juliflora* and related species) common in the southwestern United States, and cherry gum are utilized. Mesquite gum consists of L-arabinose, D-galactose and glucuronic acid in combination, and cherry gum in addition has some D-xylose and D-mannose. By controlled hydrolysis most of the pentose is removed without hydrolyzing the other constituents to any great extent. The L-arabinose is then separated from the unhydrolyzed gum and calcium salts by extraction with hot ethyl alcohol and is obtained in a crystalline condition by evaporation of the alcoholic extracts. Wheat and rye bran, peach gum, Australian black wattle gum and beet pulp have been utilized for the preparation of arabinose.

General Discussion. Although calcium chloride compounds of both the alpha and beta isomers have been crystallized,[41] only one crystalline isomer of the sugar itself is known, and this has been usually designated as the beta isomer, following the nomenclature of Hudson. The configuration for the carbons composing the pyranose ring of β-L-arabinose is the same as for α-D-galactose, as illustrated by the following formulas.

[39] P. A. Wells, J. J. Stubbs, L. B. Lockwood and E. T. Roe, *Ind. Eng. Chem.*, **29**, 1385 (1937) (see also p. 131).
[40] T. S Harding, *Sugar*, **24**, 656 (1922); E. Anderson and L. Sands, *J. Am. Chem. Soc* , **48**, 3172 (1926); "Organic Syntheses", Coll. Vol. *1*, p. 60; John Wiley & Sons, New York (1932).
[41] W. C. Austin and J. P. Walsh, *J. Am. Chem. Soc.*, **56**, 934 (1934); J. K. Dale, *ibid* , **56**, 932 (1934); H. S Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).
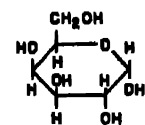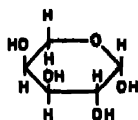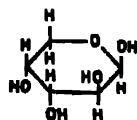
α D-Galactose   β-l Arabinose   β-D-Arabinose

This correspondence of D-galactose to L- and not D-arabinose explains many apparent anomalies exhibited by arabinose. Thus, the stable triacetylarabinosyl halides are known as beta isomers in contrast to most other acetylglycosyl halides which are the alpha isomers. Similarly, the Koenigs-Knorr synthesis results in the formation of the α-arabinosides when the known halides are treated with alcohols and silver carbonate.

D-Arabinose

For identification, properties, etc., see those of the enantiomorphic L-arabinose. The sugar is not fermentable.

Occurrence. The sugar is encountered infrequently. Cathartic-acting glycosides (aloins) such as barbaloin, isobarbaloin, nataloin and homonataloin from plants of the genus *Aloe* (*A. barbadensis*) yield D-arabinose.[42] The glycosidic union is very resistant to hydrolysis. The D-arabinose has also been reported as a constituent of tubercle bacilli.

Preparation. The D-arabinose has the same configuration as the lower five carbon atoms of D-glucose. Therefore, any of the methods for removing carbon 1 from D-glucose leads to D-arabinose or a derivative. Probably the most convenient method is the oxidation of the easily obtained calcium gluconate by hydrogen peroxide and ferric sulfate.[43]

The resolution of D,L-arabinose into the component sugars is accomplished by fractional crystallization of the active menthylhydrazones.[44]

D-Ribose
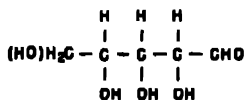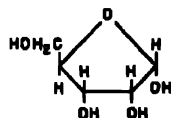
(aldehydo D Ribose)   (α D-Ribofuranose)

Properties. m.p., 87°C.; $[\alpha]_D^l = -23.1 \rightarrow -23.7$ (H_2O; c, 4; complex mutarotation). The L-isomer shows $[\alpha]_D^{20} = +20.3 \rightarrow +20.7$ (H_2O; c, 4).

Identification. Benzylphenylhydrazone, p-bromophenylhydrazone.

[42] M E. Léger, *Ann chim*, [9] 8, 265 (1917); C. S. Gibson and J L. Simonsen, *J. Chem. Soc.*, 553 (1930).

[43] R C. Hockett and C. S Hudson, *J. Am. Chem Soc.*, 56, 1632 (1934).

[44] C. Neuberg, *Ber.*, 36, 1194 (1903).

Occurrence. D-Ribose and 2-desoxy-D-ribose comprise the carbohydrate constituents of nucleic acids, which are found in all plant and animal cells. The ribonucleic acids appear to occur in the cytoplasm and the desoxyribose nucleic acids in the nucleus (Chapter IX).

Preparation.[15, 16] Although D-ribose may be synthesized from D-arabinose by the alkaline rearrangement or the glycal synthesis, the best methods start with yeast nucleic acid. The method of Levene and Clark which requires the action of ammonia at elevated temperatures and pressures has been greatly improved by Phelps who uses magnesium oxide as the hydrolytic agent. The hydrolytic products, consisting of a mixture of nucleosides, are allowed to crystallize and guanosine (guanine *N*-riboside) separates. The mother liquors are treated with picric acid and adenosine picrate (picric acid addition compound of adenine *N*-riboside) crystallizes. These nucleosides are hydrolyzed by acids, and after removal of the aglycon, the ribose crystallizes from the mother liquor.

A similar method is based on the enzymic hydrolysis of the yeast nucleic acid.[17] Emulsins prepared from sweet almonds, alfalfa seeds and many sprouted seeds hydrolyze polynucleotides (nucleic acids) to the nucleosides. Guanosine is produced almost quantitatively and adenosine picrate is likewise obtained in high yield. As in the earlier methods, the nucleosides are hydrolyzed by acids to give D-ribose.

General Discussion. The universal occurrence of D-ribose in all living cells should make this sugar of the greatest interest to biochemists and biologists. Not only is it a constituent of the nucleic acids but also of several vitamins and coenzymes (Chapter IX). This sugar and D-fructose are also unique in appearing in natural products as the furanosides. Solutions of ribose probably contain considerable quantities of the furanose form, and the mutarotation is complex and exhibits a minimum.[18]

D-Xylose
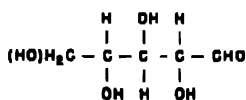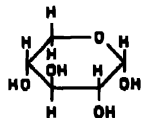
(aldehydo-D Xylose)   (α D Xylopyranose)

Synonyms. Wood sugar; in earlier literature *l*-xylose.

[15] F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars", Circular C440 of the National Bureau of Standards, Washington, D. C. (1942).

[16] P. A. Levene and E. P. Clark, *J. Biol. Chem.*, **46**, 19 (1921); F. P. Phelps, U. S Patent 2,152,662; L. Laufer and J. Charney, U. S. Patent 2,379,913, July 10, 1945.

[17] H. Bredereck, M Köthnig and E. Berger, *Ber.*, **73**, 956 (1940).

[18] F. P. Phelps, H. S Isbell and W. W. Pigman, *J. Am. Chem. Soc.*, **56**, 747 (1931). H. S Isbell and W W. Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Properties. m.p., 145°C.; $[\alpha]_D^{20} = +93.6 \rightarrow +18.8$ (H$_2$O; c, 4).
Not fermentable by ordinary yeasts.

Identification. Cadmium bromide double salt of cadmium xylonate (Cd-(C$_5$H$_9$O$_6$)$_2$·CdBr$_2$·2H$_2$O) is formed by the bromine oxidation of xylose in the presence of cadmium carbonate; phenylosazone; the hydrazones are more soluble than those from L-arabinose; monobenzylidene dimethyl acetal.

Occurrence. Polysaccharides containing xylose frequently accompany cellulose in plants and are constituents of the hemicellulose fraction. Many plant gums and several disaccharides from glycosides yield D-xylose on hydrolysis.

Preparation.[48] [49] The sugar is prepared from corn cobs (or many other woody materials) by boiling with acids, fermenting out the glucose with yeasts and crystallizing the D-xylose from the evaporated solution.

General Discussion. The presence of combined D-xylose in considerable quantities in many important agricultural wastes has stimulated interest in this sugar and its preparation. Cotton seed hulls, pecan shells, corn cobs and straw have been investigated as sources of the sugar, and several large-scale preparations[50] [51] have been carried out. The sugar crystallizes fairly easily and could be made cheaply, but insufficient uses have been developed to make the manufacture of the sugar of commercial interest. Since it is not fermentable by ordinary yeasts or utilizable by many animals, the value of the sugar is considerably limited. Sheep are able to make use of 94 to 100 per cent of ingested xylose although hogs eliminate 30 per cent in the urine. The assimilation is greater when the sugar is fed along with large amounts of other materials.[51] This pentose is cataractogenic to young rats when fed in large quantities (see under D-Galactose). Many bacteria and certain yeasts are able to ferment the sugar with the formation of important substances. Lactic and acetic acids in yield of 85 to 96 per cent are formed[52] by the action of certain *Lactobacilli* on D-xylose. *Torula* and *Monilia* yeasts grow well on hydrolyzed straw and corn cobs and provide a good cattle feed.[51]

<div align="center">

L-Xylulose

</div>

$$\begin{array}{ccccc}
& OH & H & & \\
& | & | & & \\
(HO)H_2C & C- & C - & C- & CH_2(OH) \\
& | & | & \| & \\
& H & OH & O &
\end{array}$$
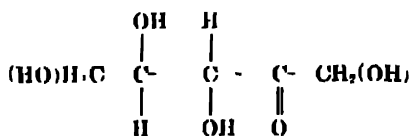
[48] T. S. Harding, *Sugar*, **25**, 124 (1923); C. S. Hudson and T. S. Harding, *J. Am. Chem. Soc*, **40**, 1601 (1918), K. P. Monroe, *ibid*, **41**, 1002 (1919)

[49] W. T. Schreiber, N. V. Geib, B. Wingfield and S. F. Acree, *Ind. Eng. Chem*, **22**, 497 (1930)

[51] N. A. Sytchev, *Compt. rend. acad. sci. U. S. S. R.*, **29**, 384 (1940)

[52] M. Iwasaki, *J. Agr. Chem. Soc. Japan*, **16**, 148 (1940).

Synonyms. L-Xyloketose, L-lyxoketose, L-*threo*-ketopentose, urine pentose, *d*-xylulose (incorrect).

Properties. $[\alpha]_D^{20} = +33.1$ (H_2O; *c*, 2); amorphous.

Identification. Phenylosazone, *p*-bromophenylhydrazone.

Occurrence. In urine of many cases of pentosuria.

General Discussion. The occasional presence of pentoses in urine was known for a considerable time before the identification of the sugar as L-xylulose by Levene and LaForge.[13] The precursor of the pentose is believed to be D-glucuronic acid since administration of this substance induces the appearance of L-xylulose in the urine.[14] Rats exhibit a significant increase of liver glycogen when fed D-xylulose but not when fed the natural L-xylulose. The natural isomer is partially utilized by dogs, however.[15]

This pentose is synthesized[16] by boiling L-xylose with pyridine, condensing the reaction products with acetone and fractionally distilling the mixture of isopropylidenexylose, lyxose and xylulose. The monoisopropylidene-L-xylulose crystallizes easily and is converted to the sugar by acid hydrolysis. This ketopentose is not capable of forming pyranose derivatives.

c. METHYLOSES

(METHYLPENTOSES)
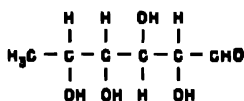
6-Desoxy-D-glucose
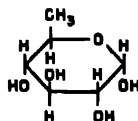
(*aldehydo*-6-Desoxy-D-glucose)   (α-6-Desoxy-D-glucopyranose)

Synonyms. D-Glucomethylose, D-isorhamnose, isorhodeose, D-epirhamnose, quinovose (chinovose).

Properties. m.p., 139–140°C.; $[\alpha]_D^{20} = +73.3 \rightarrow +29.7$ (H_2O; *c*, 8).

Identification. Phenylosazone, *p*-bromophenylosazone, 5-methylfurfural.

Occurrence and Preparation.[17] The bark of many species of *Cinchona* con-

[13] P. A. Levene and F. B. LaForge, *J. Biol. Chem.*, *18*, 319 (1914); I. Greenwald, *ibid.*, *88*, 1 (1930).

[14] M. Enklewitz and M. Lasker, *J. Biol. Chem.*, *110*, 443 (1935).

[15] H. W. Larson, N. R. Blatherwick, P. J. Bradshaw and S. D. Sawyer, *J. Biol. Chem.*, *117*, 719 (1937); H. W. Larson, W. H. Chambers, N. R. Blatherwick, M. E. Ewing and S. D. Sawyer, *ibid.*, *129*, 701 (1939).

[16] L. v. Vargha, *Ber.*, *68*, 18 (1935).

[17] C. Liebermann and F. Giesel, *Ber.*, *16*, 935 (1883); E. Fischer and C. Liebermann, *Ber.*, *26*, 2415 (1893); K. Freudenberg, *Ber.*, *62*, 373 (1929).

tains a glycoside (quinovin or chinovin) which is extracted with the quinine alkaloids. Upon treatment with alcoholic hydrogen chloride, the ethyl 6-desoxyglucoside is obtained. Convolvulin is a mixture of glycosides, one of which yields 6-desoxyglucose on hydrolysis[55] (see under D-Fucose) as does purginic acid.

L-Fucose
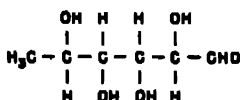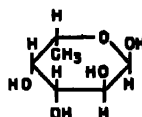
<div style="text-align:center">(aldehydo L-Fucose)   (α-L-Fucopyranose)</div>

Synonyms. L-Galactomethylose, L-rhodeose, 6-desoxy-L-galactose.

Properties. α-Isomer; m.p., 145°C.; $[\alpha]_D^{20} = -152.6 \rightarrow -75.9$ (H_2O; c, 4). Not fermentable.

Identification. p-Bromophenylhydrazone, phenylhydrazone, α-methylphenylhydrazone, 5-methylfurfural.

Occurrence. The sugar is found as a constituent of the cell walls of marine algae (sea weed) and of a few gums.

Preparation.[59, 45] Sea weed (*Fucus* species or *Ascophyllum nodosum*) is hydrolyzed by acids and the neutralized hydrolyzate fermented by galactose-acclimatized yeasts. The solution after evaporation is extracted with alcohol; after removal of the alcohol, the extracted material is converted to the difficultly soluble phenylhydrazone. The hydrazine groups are then removed by reaction with benzaldehyde and the sugar is crystallized from the liquid. The fermentation removes the mannose and galactose which often accompany the L-fucose in sea weeds. The mannose is particularly objectionable since it also forms a difficultly soluble phenylhydrazone.

D-Fucose

For formula, identification and properties see the enantiomorphous L-Fucose.

Synonyms. 6-Desoxy-D-galactose, D-galactomethylose, rhodeose.

Occurrence and Preparation.[60] This sugar is occasionally found in the hydrolytic products of glycosides. The roots of certain South and Central

[55] E. Votoček, *Ber*, **43**, 476 (1910)

[59] E. P. Clark, *J Biol Chem*, **54**, 65 (1922), R. C. Hockett, F. P. Phelps and C. S. Hudson, *J Am Chem Soc*, **61**, 1658 (1939)

[60] E. Votoček and F. Valentin, *Collection Czechoslov Chem Comm*, **1**, 46, 606 (1929); F. B. Power and H. Rogerson, *J Chem Soc*, **101**, 1 (1912); L. A. Davies and R. Adams, *J Am Chem Soc*, **50**, 1719 (1928), C. Mannich and P. Schumann, *Arch Pharm*, **276**, 211 (1936)

American plants (*Convolvulaceae*), used as purgatives, give resins of a glycosidic nature. Jalap resin (convolvulin) and Scammonium or Tampico-jalap (jalapin) are obtained from *Tubera jalapae* and *Ipomoea orizabensis*, respectively. Jalapin yields glucose, rhamnose, D-fucose and (dextro) 11-hydroxyhexadecanoic acid on hydrolysis. Convolvulin on the other hand gives among other products, 3,12-dihydroxyhexadecanoic acid, glucose, rhamnose and the 6-desoxyglucose rather than D-fucose.

L-Rhamnose
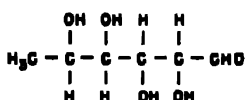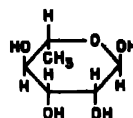
(aldehydo L-Rhamnose)   (α L-Rhamnopyranose)

Synonyms. L-Mannomethylose, 6-desoxy-L-mannose, "isodulcit"

Properties. α-Isomer (monohydrate), m.p., 93-94°C.; $[\alpha]_D^{20} = -8.6$ → $+8.2$ (H_2O; *c*, 4).

β-Isomer; m.p., 123-125°C.; $[\alpha]_D^{20} = +38.1$ → $+8.9$ (H_2O).

Not fermentable by yeasts.

Identification. β-Naphthylhydrazone, *p*-nitrophenylhydrazone, *p*-tolyl-hydrazone, phenylhydrazone, phenylosazone, 5-methylfurfural.

Occurrence.[51] The sugar is a frequent constituent of glycosides which provide its best source. It may occur[52] in the free state in the leaves and blossoms of the poison ivy, *Rhus toxicodendron* L. Some polysaccharides of gums and mucilages contain L-rhamnose.

Preparation.[53, 45] "Lemon flavin," a khaki dyestuff obtained from the bark of an oak species (*Quercus tinctoria*, Mich.), provides an excellent source of the sugar. The lemon flavin is hydrolyzed by boiling it with acids, and, after neutralization of the solution and treatment with a considerable quantity of decolorizing carbon, the sugar crystallizes from the evaporated solution.

The glycoside naringin, prepared easily from grapefruit canning wastes, has also been suggested as a source of L-rhamnose.[54]

The main constituent of the "lemon flavin" is the rhamnoside quercitrin. This glycoside yields after hydrolysis the aglycon (quercetin) and L-rhamnose. Quercitrin, a flavanol glycoside, has the following structure.[55]

[41] See: C. Liebermann and O. Hörmann, *Ann.*, 196, 299 (1879)

[42] S. F. Acree and W. A. Syme, *Am. Chem. J.*, 36, 309 (1906).

[43] T. S. Harding, *Sugar*, 25, 23, 82 (1923); C. F. Walton, *J. Am. Chem. Soc.*, 43, 127 (1921)

[44] G. N. Pulley and H. W. von Loesecke, *J. Am. Chem. Soc.*, 61, 175 (1939).

[45] See: G. F. Attree and A. G. Perkin, *J. Chem. Soc.*, 234 (1927).
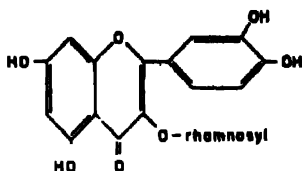
Quercitrin

General Discussion. The sugar is known in both α- and β-forms. Under ordinary conditions, the α-L-rhamnose·H_2O crystallizes. Anhydrous acetone solutions seeded with crystals of the beta isomer, crystallize giving β-L-rhamnose (anhydrous). Seed crystals of the beta isomer are obtained by melting the rhamnose hydrate and allowing the melt to crystallize at high temperatures. A molecular compound, β-rhamnose·α-rhamnose, is reported.[6a]

d. HEPTOSES

D-Mannoheptulose

$$(HO)H_2C-C\overset{H}{\underset{OH}{|}}-C\overset{H}{\underset{OH}{|}}-C\overset{OH}{\underset{H}{|}}-C\overset{OH}{\underset{H}{|}}-C-CH_2(OH)$$
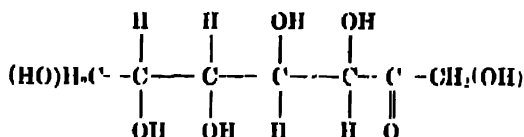
Synonyms. D-Mannoketoheptose.

Properties. m.p., 152°C.; $[\alpha]_D^{20}$ = +29.0 (H_2O). Not fermentable.

Identification. p-Bromophenylhydrazone, phenylosazone.

Occurrence. The sugar is found free accompanied by the corresponding alcohol, perseitol, in the avocado or alligator pear (*Persea gratissima*).

Preparation.[57] Ground avocados are extracted with water and the extracts evaporated to a thick sirup from which the sugar and perseitol are extracted with alcohol. The process may be repeated several times. The perseitol crystallizes from the alcoholic solution and the D-mannoheptulose is separated as the p-bromphenylhydrazone which is converted to the sugar by treatment with benzaldehyde. When crystals are once available, the sugar may be crystallized directly from the extracts.

General Discussion. Studies of the physiological availability of the sugar exhibit an interesting species difference. Rabbits can utilize D-mannoheptulose but rats cannot. The related aldoheptose α-mannoheptose is not utilized by either species.[58]

A number of derivatives of the sugar have been prepared.[59]

[66] E. L. Jackson and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 1076 (1937); E Fischer, *Ber.*, **28**, 1162 (1895); T. Purdie and C. R. Young, *J. Chem. Soc.*, **89**, 1194 (1906)

[57] F. B. LaForge, *J. Biol. Chem.*, **28**, 517 (1916).

[58] J. H. Roe and C S. Hudson, *J. Biol Chem.*, **121**, 37 (1937)

[59] E. M. Montgomery and C S. Hudson, *J Am Chem. Soc .*, **61**, 1654 (1939)

The constitution of the sugar was shown by conversion to an osazone which is identical with that obtained from the aldoheptoses produced from mannose by the cyanohydrin synthesis.

Sedoheptulose

$$\underset{OH}{\overset{H}{\underset{|}{\overset{|}{(HO)H_2C—C}}}}\underset{OH}{\overset{H}{\underset{|}{\overset{|}{—C}}}}\underset{OH}{\overset{H}{\underset{|}{\overset{|}{—C}}}}\underset{H}{\overset{OH}{\underset{|}{\overset{|}{—C}}}}\underset{O}{\overset{}{\underset{\|}{\overset{}{—C}}}}—CH_2(OH)$$
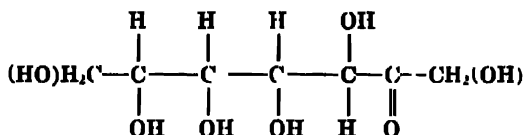
Synonyms. D-Altroheptulose; possibly identical with volemose and volemulose.

Properties. Amorphous; $[\alpha]_D = +2$ to 3 (H_2O; c, 10). Not fermentable by yeasts.

Identification. Phenylosazone, p-bromophenylosazone, formation of sedoheptulosan on treatment with acids.

Occurrence. Found originally in *Sedum spectabile*, Bor., a common, herbaceous, perennial plant used for decorative purposes.[70] The presence of the free sugar in other *Sedum* species has also been noted [71]

Preparation.[72, 70] The sugar is extracted by water from ground *Sedum* leaves and stems and the extracts are evaporated to a thick sirup. The sedoheptose is extracted by alcohol which is removed by evaporation. An aqueous solution of the sirup is purified with lead acetate. After removal of the excess lead by precipitation with hydrogen sulfide, a crude solution of the sugar is obtained.

General Discussion. Sedoheptulose has a pyranose ring of the D-altrose type, *i.e.*, the hydrogen atom of carbon 1 of D-altrose may be considered to be replaced by a CH_2OH group, and is the sole known representative of the altrose type which is found in nature (see Digitoxose, however). In agreement with the similar property of D-altrose, it is converted in acid solution to an anhydro derivative, sedoheptulosan. The available evidence[71] indicates that the anhydro derivative has the unique structure of 2,3-anhydro-sedoheptuloheptanose with 2,3 and 2,6 rings.

The sugar has been suggested as a source for D-altrose and D-ribose since it is easily oxidized by oxygen in alkaline solution to D-altronic acid; calcium altronate is oxidized by hydrogen peroxide and ferric acetate to D-ribose. These reactions also provide proof for the assignment of the D-altrose configuration to the sugar.[73]

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[70] F. B. LaForge and C. S. Hudson, *J. Biol. Chem*, *30*, 61 (1017).

[71] M. Proner, *Bull. sci. pharmacol*, *43*, 7 (1936)

[72] N. K. Richtmyer, R. M. Hann and C. S. Hudson, *J. Am. Chem Soc*, *61*, 343 (1939).

[73] C. S. Hudson, *J. Am. Chem. Soc.*, *60*, 1241 (1938).

2. Synthetic Sugars

A. Complete Synthesis of the Sugars. The total synthesis of glucose, fructose and mannose from "coal, air and water" has been achieved, but as practical methods the processes cannot compete with the biochemical production of sugars by plants from carbon dioxide and water. The principal difficulty in the chemical synthesis is the separation of the great number of isomers which are formed when inactive substances are condensed by inactive agents. In the biological synthesis, the catalytic agents are enzymes which, as asymmetric agents, guide the reactions in certain directions so that only certain isomers are formed. The over-all reaction may be represented:

$$6CO_2 + 6H_2O \xrightarrow{\text{chlorophyll}} 6O_2 + C_6H_{12}O_6 - 673,000 \text{ cal.}$$
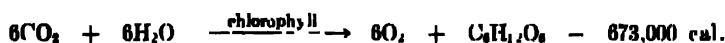
According to the theory of von Baeyer, the photosynthesis of carbohydrates in plants may take place through the intermediate formation of formaldehyde. It is of interest then that formaldehyde condenses in the presence of weak bases to form a complex mixture of sugars called formose or methose.[74]
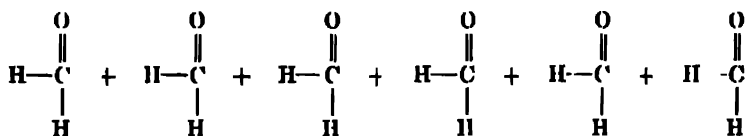
(Formaldehyde)
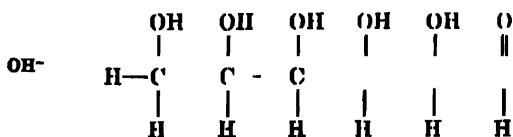
(Hexoses)

Although often termed an aldol condensation, the above reaction differs from the true aldol condensation which takes place between the carbonyl group of one molecule and an α-hydrogen of another molecule. The reaction does not take place as indicated above but instead proceeds through the various possible intermediates: glycolaldehyde, glyceraldehyde and dihydroxyacetone. Ketohexoses are formed by the combination of glyceraldehyde and dihydroxyacetone, and ketopentoses from glycolaldehyde and dihydroxyacetone.[75] For the lower sugars, the direct synthesis from

[74] A. Butlerow, *Ann.*, *120*, 295 (1861); O. Loew, *J. prakt. Chem.*, [2] *33*, 321 (1886).

[75] H. and A. Euler, *Ber.*, *39*, 45 (1906); L. Orthner and E. Gerisch, *Biochem. Z.*, *259*, 30 (1933).

Jormaldehyde may be the best method of preparation because the number of isomers is not so great as when the carbon chains are longer [76]

Formose (and similar products obtained by the condensation of acrolein dibromide and of glyceraldehyde under the influence of dilute alkali) when treated with phenylhydrazine gives two crystalline osazones in yields of

Fischer's Complete Synthesis of Hexose Sugars

α-Acrose (from formaldehyde, glyceraldehyde or acrolein dibromide)

$\downarrow$ C₆H₅NHNH₂.

D,L Glucosazone (α phenylacrosazone)

$\downarrow$ conc HCl

D,L-Glucosone (α acrosone)

$\downarrow$ Zn, HOAc

D L-Fructose   ──fermentation with yeast──→   L-Fructose

$\downarrow$ Na Hg (reduction)

D,L Mannitol ← ──Na Hg──   D,L Mannose (L mannose made from L arabinose)

$\downarrow$ HNO₃ oxidation, purification through phenylhydrazone

D,L Mannose   ──fermentation with yeast──→   L Mannose

$\downarrow$ HₙO oxidation

D,L-Mannonic acid

$\downarrow$ Separated by fractional crystallization of strychnine salts

D-Mannonic acid ──Na–Hg──→ D-Mannose   L Mannonic acid

$\downarrow$ Heat with quinoline

D-Gluconic acid

$\downarrow$ Na Hg (reduction)

D-Glucose   ──C₆H₅NHNH₂──→   D-Glucosazone

$\downarrow$ conc HCl

D-Glucosone

$\downarrow$ Zn, HOAc

D-Fructose

---

[76] E. J. Lorand, U. S. Patent 2,272,378, Feb. 10, 1942

about 13 per cent.[77,][78] The two osazones were given the name α-acrosazone and β-acrosazone; the corresponding sugar components are α-acrose and β-acrose. The constitution of these products was demonstrated in an outstanding series of researches by Fischer and Tafel[79] which also led to the complete synthesis of D-glucose, D-mannose and D-fructose. (See page 112.)

From this evidence, particularly the identification of the D,L-mannitol from natural sources with that from the α-acrosazone, the α-acrose may be any (or all) of the three D,L-sugars which yields the D,L-glucosazone: glucose, mannose and fructose. The β-acrosazone has been shown to be D,L-sorbosazone which would be produced from gulose and idose as well as sorbose.[78,][80] Considerations of the mechanism of the reaction indicate that only the ketoses can result from the reactions of the glyceraldehyde and of the acrolein dibromide, and the resistance of formose to bromine oxidation supports this view. The condensations of these products are undoubtedly aldol condensations which take place between the carbonyl groups and hydrogen atoms *adjacent* to carbonyl groups. If two molecules of glyceraldehyde reacted in this fashion the product would have a branched chain and not a straight chain.[81] However, in alkaline solution glyceraldehyde is in equilibrium with dihydroxyacetone, and these substances could condense to form ketoses. The similar behavior of the acrolein dibromide is ascribed to the formation of glyceraldehyde by substitution of the bromine atoms by hydroxyl groups. The probable reaction is represented by the following equation:

| Acrolein dibromide | D, L-Glyceraldehyde | Dihydroxy acetone | | Ketohexose |
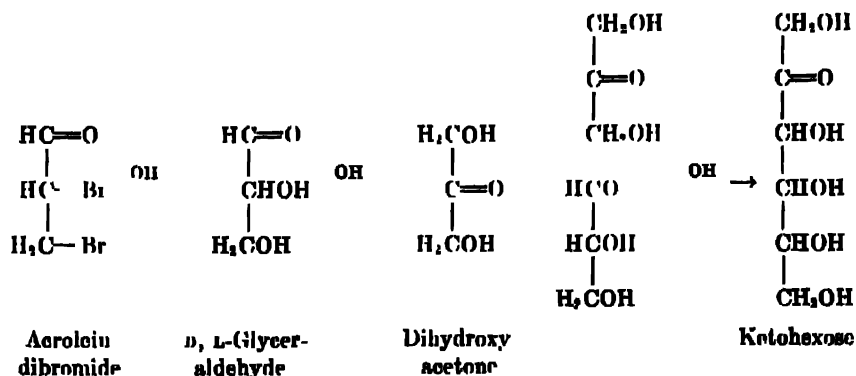
This mechanism is substantiated by the direct isolation from the acrose

[77] E. Fischer and J. Tafel, *Ber.*, *20*, 2566 (1887).

[78] W. Küster and F. Schoder, *Z. physiol. Chem.*, *141*, 110 (1924).

[79] See summary by E. Fischer, *Ber.*, *23*, 2114 (1890).

[80] F. Schmitz, *Ber.*, *46*, 2327 (1913).

[81] This may be an explanation of the formation of the few natural branched-chain sugars: apiose and hamamelose.

mixture of D,L-sorbose.[90] Of the four possible ketohexoses which should be formed since no asymmetric reagents are involved, two have been identified as α-acrose (D,L-fructose) and β-acrose (D,L-sorbose). The other isomers are undoubtedly to be found in the condensation products along with many other substances.

The number of isomers formed may be greatly reduced by utilizing the optically active D-glyceraldehyde and dihydroxyacetone. When these substances are condensed by 0.01 M barium hydroxide solution, a mixture of D-fructose and D-sorbose is obtained.[92] The other two possible ketohexoses (D-tagatose and D-psicose) are not formed in detectable quantities. The asymmetric synthesis favors the formation of *trans* hydroxyl groups for the two new asymmetric centers (carbons 3 and 4). Branched-chain sugars are formed under these conditions from 2,3-isopropylidene-D-glyceraldehyde.
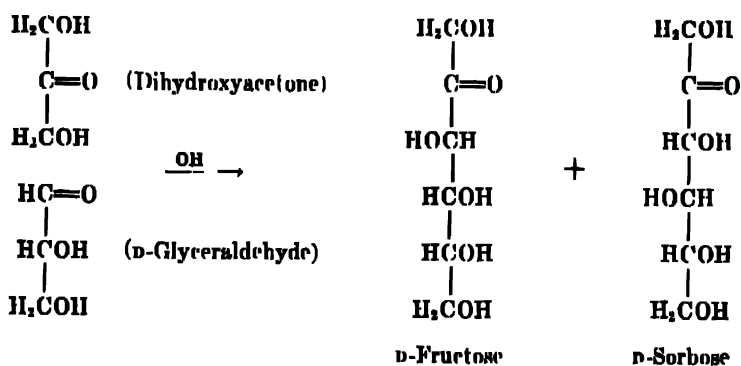
The employment of an asymmetric catalyst (enzyme) directs the course of the reaction so that still fewer isomers are formed. Thus, D-glyceraldehyde and the dihydroxyacetone 1-phosphate condense in the presence of an enzyme, aldolase from yeast and muscle extracts, giving D-fructose 1-phosphate as the sole reaction product.[83] Fermentable sugars are also formed directly from dihydroxyacetone by liver enzymes, but some nonfermentable as well as fermentable sugar is obtained from r-glyceraldehyde. The fermentable sugar is probably glucose and the nonfermentable ketose sorbose.[84]

Partially methylated sugars may be prepared from the corresponding methylated polymerizing substances. Thus, methoxyacetaldehyde, pre-

[81] H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, 19, 519 (1936).
[83] O. Meyerhof, K. Lohmann and P. Schuster, *Biochem. Z.*, 286, 319 (1936).
[84] See: H. Imanaga, *Biochem. Z.*, 294, 342 (1937).

pared by chromic acid oxidation of methoxyethanol, polymerizes in the presence of potassium carbonate to 2,4-dimethylaldotetrose [85a]

$$2CH_3OCH_2—CHO \xrightarrow{K_2CO_3} CH_3OCH_2—CH(OH)—CH(OCH_3)—CHO$$

Direct chemical syntheses of the D,L-aldotetroses (D,L-threose and D,L-erythrose) have been devised [85b] The syntheses are outlined below:

a) D,L Threose

$$CH_2Cl \ CH(OH) \ CH_2Cl \xrightarrow{KCN} CH_2Cl \ CH(OH) \ CH_2 \ CN \xrightarrow[C_2H_5OH \ HCl]{H_2O} \rightarrow$$

(1,3-Dichloro 2 propanol)

$$CH_2Cl \ CH(OH) \ CH_2 \ COOC_2H \xrightarrow{P_2O_5} CH_2Cl \ CH=CH \ COOC_2H \xrightarrow[OH]{H_2O} \rightarrow$$

$$CH_2Cl \ CH=CH \ COOH \xrightarrow[OsO_4]{Ba(ClO_3)_2} CH_2Cl \ CH(OH) \ CH(OH) \ COOH \xrightarrow[C_2H_5OH]{KOH} \rightarrow$$

$$potassium \ salt \xrightarrow{heat} \begin{array}{c} \overbrace{\qquad\qquad O \qquad\qquad} \\ CH \ CH(OH) \ CH(OH) \ CO \end{array} \xrightarrow{NH_3} \rightarrow$$

(D, L Threonic lactone)

$$(HO)H \ C \ CH(OH) \ CH(OH) \ CONH_2 \xrightarrow[C_6H_5N]{C_6H_5COCl} tribenzoate \xrightarrow[HOAc]{N_2O_4} \rightarrow$$

$$(BzO)H_2C \ CH(OBz) \ CH(OBz) \ COOH \xrightarrow{SOCl_2} \rightarrow$$

$$(BzO)H_2C \ CH(OBz) \ CH(OBz) \ COCl \xrightarrow{Pd \ H_2} \rightarrow$$

$$(BzO)H_2C \ CH(OBz) \ CH(OBz) \ CHO \xrightarrow{OH^-} (HO)H \ C \ CH(OH) \ CH(OH) \ CHO$$

(D,L Threose, sirup)

A better procedure for the preparation of ethyl 4-chlorocrotonate begins with allyl alcohol rather than 1,3-dichloro-2-propanol

b) D,L-*Erythrose*

$$CH_2=CH \ CH_2OH \xrightarrow[HCl]{Cu_2(CN)_2} CH_2=CH \ CH_2 \ CN \xrightarrow[HCl]{H_2O} \rightarrow$$

$$CH_2=CH \ CH_2 \ COOH \xrightarrow[OsO_4]{Ba(ClO_3)_2} \begin{array}{c} \overbrace{\qquad\qquad} \\ H \ C \ CH(OH) \ CH_2 \ CO \end{array} \xrightarrow{P_2O_5} \rightarrow$$

$$\begin{array}{c} \overbrace{\qquad O\qquad} \\ H_2C—CH=CH \ CO \end{array} \xrightarrow[OsO_4]{Ba(ClO_4)_2} \begin{array}{c} \overbrace{\qquad\qquad O \qquad\qquad} \\ H \ C—CH(OH)—CH(OH)—CO \end{array}$$
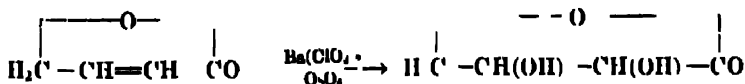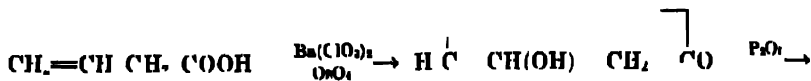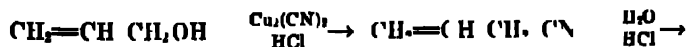
D,L Erythronic lactone

[85a] C D Hurd and J L Abernethy, *J Am Chem Soc*, 63 1966 (1941)

[85b] W W Lake and J W E Glattfeld, *J Am Chem Soc*, 66, 1091 (1944), J W E Glattfeld and B D Kribben, *ibid* 61, 1721 (1939), G Braun, *ibid*, 52, 3167 3176 (1930), 54, 1133 (1932)

The D,L-erythronic lactone was converted to the potassium salt which was acetylated. The acetyl derivative was treated with $SOCl_2$, the acid chloride was reduced catalytically to the acetylated sugar Finally, D,L-erythrose (sirupy) was obtained by alkaline deacetylation of the diacetate

Allitol has been synthesized by hydroxylation of the meso form of divinylglycol, prepared in turn from acrolein (CH_2=CH   CHO) ~ Dulcitol and D,L-mannitol were obtained in a similar manner

B. Methods for Lengthening the Carbon Chain of the Sugars. To be important, methods for increasing the length of the carbon chain of the sugars must add a CHOH rather than a CH_2 group. The well-known addition of hydrocyanic acid to ordinary aldehydes and ketones and giving cyanohydrins fulfills this condition. The cyanohydrins are hydrolyzed to

$$R-CHO + HCN \rightarrow R-\overset{\displaystyle OH}{\underset{\displaystyle H}{\overset{|}{\underset{|}{C}}}}-CN \xrightarrow{OH} R-\overset{\displaystyle OH}{\underset{\displaystyle H}{\overset{|}{\underset{|}{C}}}}-COOH + R-\overset{\displaystyle H}{\underset{\displaystyle OH}{\overset{|}{\underset{|}{C}}}}-COOH$$

Cyanohydrin
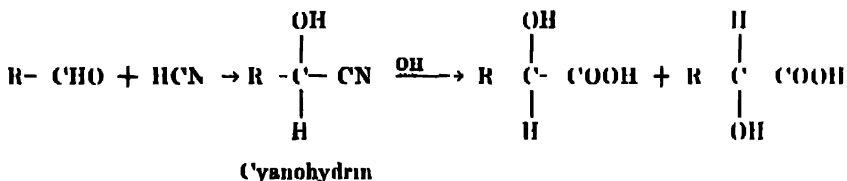
the α-hydroxyacids. The original procedure devised by Kiliani[46] for applying this reaction to the sugars involved the reaction of the sugar with an aqueous solution of hydrocyanic acid in the presence of a little ammonia This has been greatly simplified and improved by adding the sugar to a solution of sodium cyanide and calcium chloride.[47] The cyanohydrins are hydrolyzed by heating with lime or barium hydroxide, and when the solution is cool, basic salts of the aldonic acid calcium or barium salts crystallize. These may be converted to the corresponding normal calcium salts by treatment with carbon dioxide and to the free acids by sulfuric acid.

Inasmuch as a new asymmetric center is created, two isomers are formed, but since the original substance is optically active, the amounts of the two isomers will usually not be equal (asymmetric synthesis) The proportions vary from about equal amounts to almost entirely one isomer for the product obtained by the Kiliani synthesis from mannose The generalization has been made[48] that the product formed in largest quantity by the cyanohydrin synthesis has a *trans* configuration for carbons 2 and 4, but it seems

[45] See R Lespieau, *Advances in Carbohydrate Chem*, *2* 107 (1946); J Wiemann *Ann chim*, [11] 5, 316 (1936)

[46] H Kiliani, *Ber*, *19*, 3033 (1886).

[47] C S Hudson, O Hartley and C B Purves, *J Am Chem Soc*, 56, 1248 (1934)

[48] J G Maltby, *J Chem Soc.*, 1404 (1923); 1629 (1926), 2769 (1929), R M Hann \ T. Merrill and C S Hudson, *J Am Chem Soc* 66, 1912 (1944), C S Hudson *Advances in Carbohydrate Chem.*, 1, 26 (1945).

likely that the proportions obtained will depend on the conditions of the reaction.

The two acids produced are separated by fractional crystallization of the metallic or alkaloidal salts, the phenylhydrazides, amides, double salts, benzylidene derivatives, etc. The free acid is then formed and converted to the lactone by heating with dilute acids. The lactone finally is reduced to the sugar by sodium amalgam at 0°C. in slightly acid solution. When conducted in alkaline solutions, the reductions proceed further to the alcohols. It is of interest that the lactones, but not the aldonic acids, are reduced by this treatment. This reduction process though tedious has been and remains of great importance, for it was developed by Fischer in his classical work which established the stereochemical formulas of the sugars (see p. 27). Reduction to the sugars may also be accomplished by the catalytic hydrogenation of the acetylated aldonic acid chlorides or thio esters.[89] (*See also p. 299.*)

The cyanohydrin synthesis is illustrated by the preparation of the heptoses from mannose.[90]
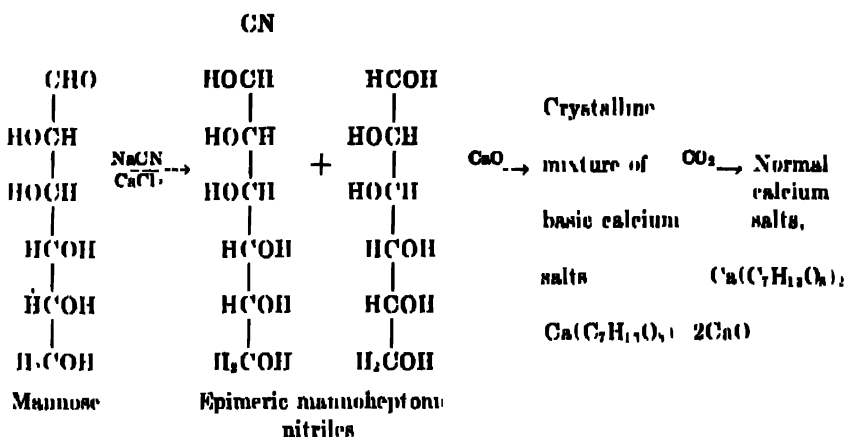
By direct crystallization of the calcium salts one isomer is obtained in fairly pure form The mother liquors which contain both isomers are converted to the free acids by treatment with sulfuric acid and finally to the lead salts by neutralization of the acids with lead oxide. Fractional crystallization of these lead salts leads to separation of the two isomers. These are converted to the lactones. (I, II), which in turn are reduced to the sugars (III, IV) by sodium amalgam in slightly acid solution.

[89] E W. Cook and R. T. Major, *J. Am. Chem. Soc*, **58**, 2410 (1936), J. W. E Glattfeld and B D Kribben, *ibid.*, **61**, 1720 (1939); M. L. Wolfrom and J. V. Karabinos, *ibid.*, **68**, 1155 (1946).

[90] H S Isbell, *J Research Natl. Bur. Standards*, **20**, 97 (1938).

The cyanohydrin synthesis should make it possible to go from the simplest member of the sugar series, such as glyceraldehyde, to all of the higher sugars although it has not been carried through the tetrose stage. Synthetic

$$
\begin{array}{cccc}
\text{CHO} & -\text{C}=\text{O} & -\text{C}=\text{O} & \text{CHO} \\
\text{HOCH} & \text{HOCH} & \text{HCOH} & \text{HCOH} \\
\text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HOCH} \\
\text{HOCH} & \text{CH} & \text{CH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{H}_2\text{COH} & \text{H}_2\text{COH} & \text{H}_2\text{COH} & \text{H}_2\text{COH} \\
(IV) & (I) & (II) & (III)
\end{array}
$$
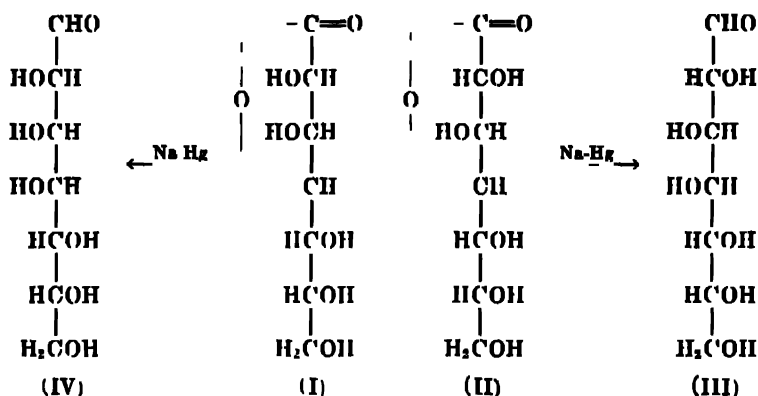
sugars with as many as ten carbons in a straight chain (e.g., D-glucodecose) have been prepared from the hexoses by this method.[91]

The reaction of the acyl chlorides, or of the open-chain free-aldehyde and ketone derivatives of the sugars, with diazomethane provides another method for the addition of a carbon atom.[92] The free-aldehyde group reacts to form methylketones (1-desoxyketoses).

$$
\text{R} - \text{CHO} \xrightarrow{\text{CH}_2\text{N}_2} \text{R}-\overset{\displaystyle O}{\overset{\|}{\text{C}}}-\text{CH}_3 + \text{N}_2
$$
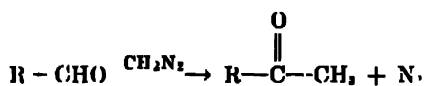
The acyl halides yield diazomethyl ketones which on hydrolysis give the hydroxymethyl ketones and on acetolysis, the corresponding acetyl derivatives.[93] Thus, D-erythrulose is formed by the hydrolysis of the diazoketone formed from isopropylidene-D-glyceryl chloride and diazomethane.

Isopropylidene
D-glyceryl chloride   D-Erythrulose
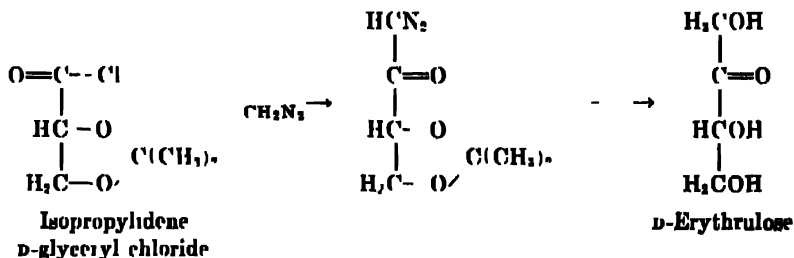
[91] L. H. Philippe, Ann. chim. phys., [8] 26, 393 (1912).

[92] P. Brigl, H. Muhlschlegel and R. Schinle, Ber., 64, 2921 (1931); M. L. Wolfrom, I. Weisblat, W. H. Zophy and S. W. Waisbrot, J. Am. Chem. Soc., 63, 201 (1941).

[93] K. Iwadare, Bull. Chem. Soc. Japan, 14, 131 (1939) See also: M. L. Wolfrom, R. L. Brown and E. F. Evans, J. Am. Chem. Soc., 65, 1021 (1943); K. Gatzi and T. Reichstein, Helv. Chim. Acta, 21, 186 (1938).

By the action of Grignard reagents on suitably blocked esters or similar derivatives of the sugar acids, alkyl or aryl groups may be added to the sugars with the formation of carbon-carbon bonds.[44a,b]

$$
\begin{array}{ccc}
\text{COOH} & \text{CO} & \text{R} \\
| & | & | \\
\text{(CH}_3\text{)}_2\text{C} & \overset{\text{RMgBr}}{\longrightarrow} & \text{(CH}_3\text{)}_2\text{C} & + & \text{R—COH}
\end{array}
$$

Diisopropylidene-
2-keto gluconic acid
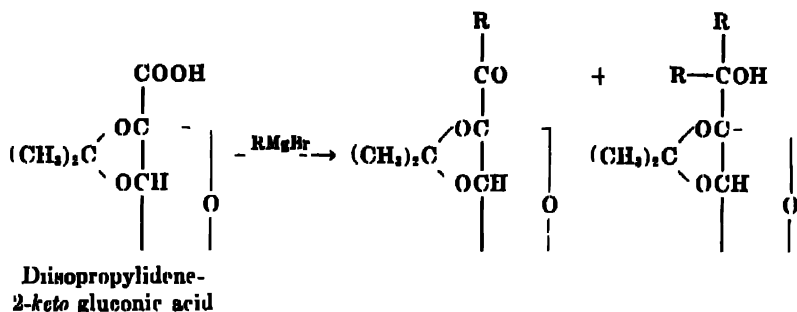
Aldehydo derivatives of the sugars in which the hydroxyls are blocked react with the Grignard reagent to give C-substituted glykitols.[44b,c]

$$
\begin{array}{ccc}
\text{R} & \text{R} & \text{R} \\
| & | & | \\
\text{HCO} & \overset{\text{RMgX}}{\longrightarrow} & \text{HCOMgX} & \overset{\text{HOH}}{\longrightarrow} & \text{HCOH} \\
| & & | & & | \\
\text{HCOR}' & & \text{HCOR}' & & \text{HCOR}'
\end{array}
$$

(R=CH₃, cyclohexyl, phenyl, α-naphthyl)
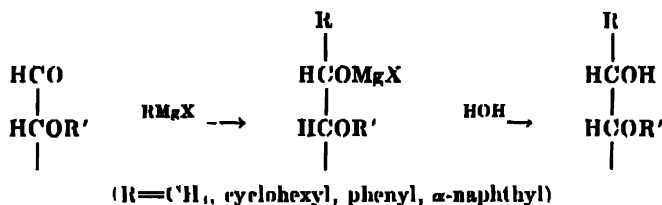
The same type of derivative is made by the reaction of glycosyl halides with the Grignard reagent.[44d] If acetyl groups are present, the carbonyl of the acetate groups react with the reagent, and sufficient reagent must be used to react with all of the acetyl groups as well as the hemiacetal halide group.

Identical derivatives are made by application of the Friedel-Crafts reaction, but by this method it is possible to proceed farther and add two hydrocarbon radicals to the carbon chain of the glycosyl halides. Thus, Hurd and Bonner[44e] found that aluminum chloride catalyzes a reaction between tetraacetyl-α-glucosyl chloride and benzene to yield 2,3,4,6-tetraacetyl-1-phenyl-1-desoxy-D-glucose (I) and 2,3,4,5,6-pentaacetyl-1,1-diphenyl-1-desoxy-D-sorbitol (II). This represents a new application of the Friedel-Crafts reaction and involves glucosylation (by the tetraacetyl-α-glucosyl chloride) of the aromatic nucleus. Because of the cleavage of

[44a] C. Paal, Ber , 49, 1583 (1916); H. Ohle et al., Ann., 481, 233, 255 (1930); 492, 1 (1931).

[44b] K. Gatzi and T. Reichstein, Helv. Chim Acta, 21, 914 (1938).

[44c] J. English, Jr , and P. H. Griswald, Jr., J. Am Chem. Soc., 67, 2039 (1945).

[44d] C. D. Hurd and W. A. Bonner, J. Am. Chem. Soc , 67, 1972 (1945).

[44e] C. D. Hurd and W. A. Bonner, J Am. Chem. Soc., 67, 1664, 1759, 1977 (1945).

the ester groups and subsequent formation of acetophenone, more than catalytic quantities of aluminum chloride are needed. The amount of catalyst present influences the composition of the reaction products; five moles favor the formation of the monosubstituted product while

```
   HC   C₆H                      C₆H₅—CH—C₆H₅
   |                                  |
   HCOAc                              HCOAc
   |                    O             |
   AcOCH                |             AcOCH
   |                    |             |
   HCOAc                              HCOAc
   |                                  |
   HC                                 HCOAc
   |                                  |
   CH₂OAc                             CH₂OAc
     (I)                               (II)
```
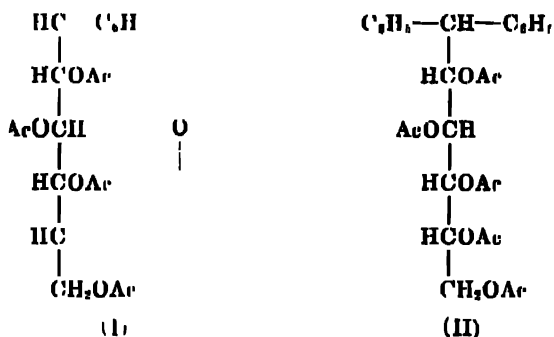
eight moles of aluminum chlorides enhance the yield of the disubstituted product. This reaction was further extended to toluene, from which the disubstituted product was the only pure compound isolated.

The monosubstituted derivative (I) yields the disubstituted derivative (II) upon treatment with benzene and aluminum chloride. By treatment of the monosubstituted compounds with an aromatic hydrocarbon different from that used originally, a new asymmetric center is created and two isomers are possible:

```
       H                              H
       |                              |
  R₁ —C—R₂          and          R₂—C—R₁
       |                              |
     HCOH                           HCOH
       |                              |
```

Compounds of this type have been prepared in which R_1 = p-tolyl and R_2 = phenyl groups.

In the presence of sodium methylate, nitromethane adds to 1,6-benzylidene-glucose to give a seven-carbon nitro alcohol,[88] which upon conversion to the sodium salt and subsequent treatment with strong acid gives D gluco-D-gulo-heptose (α-glucoheptose)

```
                              CH₂NO₂                        HCO
         HCO                   |                             |
          |          CH₃NO₂    HCOH                          HCOH
          |          CH₂ONa →   |             ----→          |
         HCOH                   HCOH                         HCOH
          |                      |                            |
```
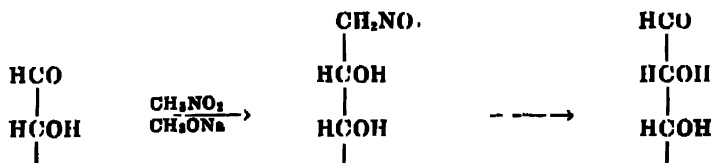
[88] J. C. Sowden and H O L. Fischer, *J. Am. Chem Soc.*, **68**, 1511 (1946).

Another method[95] is of particular value for the preparation of ascorbic acid and similar substances. The procedure depends on the condensation of sugars with the ethyl ester of glyoxylic acid ($CHO - COOC_2H_5$) in the presence of cyanides. The reaction is similar to the well-known benzoin condensation, and two carbons are added to the sugar carbon chain (p. 315). It would seem desirable that this method receive additional investigation.

```
C₂H₅O—C=O                    [⁻    C=O
    |                         |
   HCO                       COH
    |          NaCN      O    |
   HCO                       COH        (D-Ascorbic acid)
    |                         |
  HOCH                   L - —CH
    |                         |
   HCOH                      HCOH
    |                         |
  CH₂OH                      CH OH
```
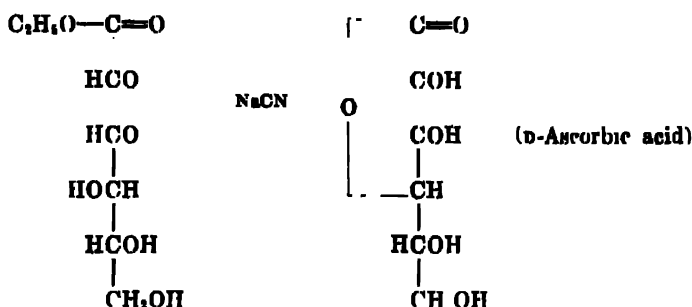
C. Methods for Shortening the Carbon Chains of Sugars.

One of the most convenient methods for shortening the carbon chains of sugars is the Ruff degradation.[96] Prior to Ruff's work, H J H. Fenton had shown that tartaric acid is oxidized by hydrogen peroxide in the presence of *ferrous* salts, but apparently no oxidative cleavage of carbon-carbon bonds was noted. The *ferrous* ion catalyzed oxidation was extended to many carbohydrates by associates of Fenton (see p. 336) and by other workers.[97] *Ferric* ions are used as the catalyst in the Ruff method. This catalyst permits the oxidation of aldonic acids but is inactive with respect to sugars. *Ferrous* ions are much less selective.

Ruff applied the reaction to the easily available salts of the aldonic acids and showed that the oxidation takes place by the cleavage of carbon-carbon bonds and the direct formation of sugars. The yields are often small, but the sugars usually crystallize readily. D-Arabinose is produced from calcium D-gluconate in 50 per cent of the theoretical quantity. However, D-lyxose is formed from calcium galactonate in only 17 per cent of the calculated amount.[98] Other salts than the calcium salts may be employed, e.g., strontium D-xylonate is oxidized to D-threose.[99]

[95] B. Helferich and O Peters, Ber , 70, 465 (1937)

[96] O. Ruff, Ber., 31, 1573 (1898); 34, 1362 (1901); O Ruff and G. Ollendorff, Ber., 33, 1798 (1900).

[97] For a discussion of the development of the reaction see: C. F. Cross, E J. Bevan and T. Heiberg, J Chem. Soc., 75, 747 (1899); R. S Morrell and J. M. Crofts, ibid., 75, 786 (1899).

[98] R. C. Hockett and C. S. Hudson, J. Am. Chem Soc., 56, 1632 (1934).

[99] R. C. Hockett, J. Am. Chem. Soc , 57, 2260 (1935).

$$
\begin{array}{ccc}
\underset{\substack{\text{Calcium} \\ \text{D-gluconate}}}{
\begin{array}{c}
\text{COO}^- \\
| \\
\text{HCOH} \\
| \\
\text{HOCH} \\
| \\
-\text{C}- \\
|
\end{array}}
& \xrightarrow[\text{Fe}^{+++}]{-\text{ H}_2\text{O}_2} &
\underset{\text{D-Arabinose}}{
\begin{array}{c}
\text{CO}_2 \\
+ \\
\text{HC}=\text{O} \\
| \\
\text{HOCH} \\
| \\
-\text{C}- \\
|
\end{array}}
\end{array}
$$
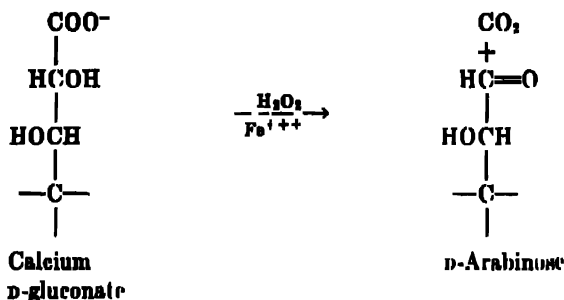
The copper salts of the aldonic acids may be degraded to sugars by electrolysis between platinum electrodes.[100]

The important degradation procedure of Wohl[101] is essentially the reverse of the cyanohydrin synthesis. It involves the removal of the cyanide group from the acetylated nitriles, which in turn are formed from the oximes by application of the usual acetylation procedures.

$$
\underset{\text{Hexose}}{
\begin{array}{c}
\text{CHO} \\
| \\
\text{CHOH} \\
| \\
-\text{C}- \\
|
\end{array}}
-\rightarrow
\underset{\text{Oxime}}{
\begin{array}{c}
\text{CH}=\text{NOH} \\
| \\
\text{CHOH} \\
| \\
-\text{C}- \\
|
\end{array}}
\xrightarrow[\substack{\text{NaOAc,} \\ \text{heat}}]{(\text{Ac})_2\text{O}}
\underset{\text{Nitrile}}{
\begin{array}{c}
\text{CN} \\
| \\
\text{CHOAc} \\
| \\
-\text{C}- \\
|
\end{array}}
-\rightarrow
\underset{\text{Pentose}}{
\begin{array}{c}
\text{HCN} \\
+ \\
\text{CHO} \\
| \\
-\text{C}- \\
|
\end{array}}
$$
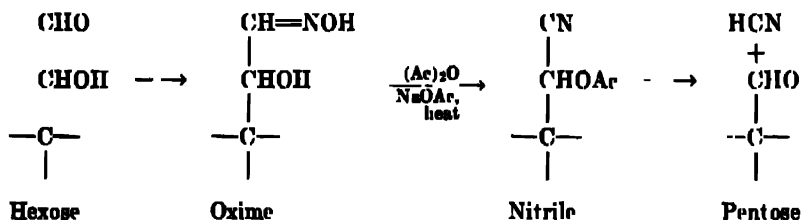
The preparation of the nitriles has been discussed under the oximes (p. 410). In the original procedure, the hydrocyanic acid was eliminated by the action of ammoniacal silver oxide or sodium hydroxide, but sodium methylate in chloroform solution later was employed.[102] The several modifications of the method have been critically studied by Deulofeu,[103] who suggests the use of the Zemplén modification for the hexoses and higher sugars and the original procedure for the lower sugars. Later studies[104] have shown the superiority of strong ammonia (28 per cent) for the degradation step. The diacetamide derivatives which are formed are converted to the free sugars by acid hydrolysis.

[100] C. Neuberg, *Biochem. Z.*, 7, 527 (1907).

[101] A. Wohl, *Ber.*, 26, 730 (1893).

[102] G. Zemplén and D. Kiss, *Ber.*, 60, 165 (1927).

[103] V. Deulofeu, *J. Chem. Soc.*, 2602 (1930).

[104] R. C. Hockett, V. Deulofeu, A. L. Sedoff, and J. R. Mendive, *J. Am. Chem. Soc.*, 60, 278 (1938).

```
CN
|
HCOAc                                                            CHO
|                        HC—(NHAc)₂                              |
HCOAc    NH₄OH⟶          |              H⁺⟶                      HCOH
|                        HCOH                                    |
                         |

Nitrile                  Diacetamide                             Sugar
(Hexose)                 (Pentose)                               (Pentose)
```
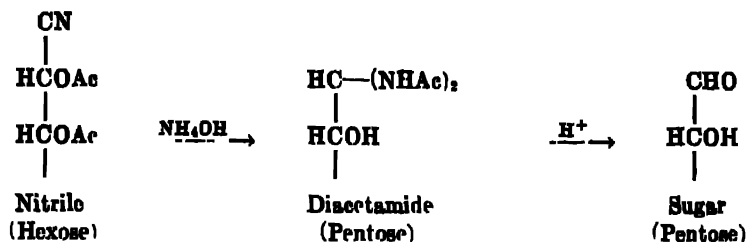
The Hofmann method for the degradation of amides to the amines of one less carbon, was applied by Weerman[105] to the amides of α-hydroxy-acids and led to the production of aldehydes with one carbon less than the original acid.

```
COOH           CONH₂                  ⎡  N:CO  ⎤           HNCO
|              |                      ⎢   |    ⎥             +
CHOH    NH₃⟶   CHOH      NaClO⟶       ⎢  CHOH  ⎥    ⟶       CHO
|              |                      ⎢   |    ⎥             |
–C–            –C–                    ⎢  –C–   ⎥            –C–
|              |                      ⎣   |    ⎦             |

Hexose         Amide                  Isocyanate            Pentose
aldonic
acid
```
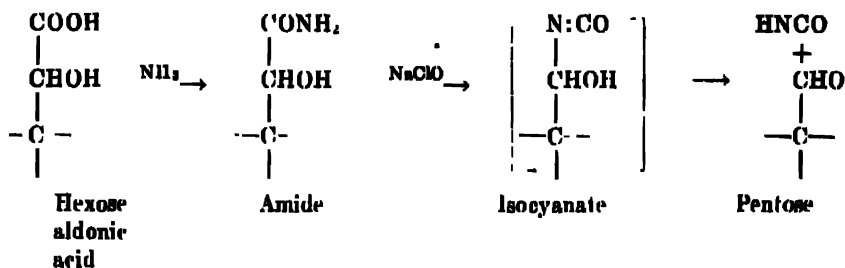
In addition to its value as a synthetic method, the reaction is also of value for demonstrating the presence of a hydroxyl on the carbon adjacent to the carboxyl group. Although sodium isocyanate is liberated when carbon 2 carries a free hydroxyl, a cyclic urethane (I) (readily convertible to the lower sugar, II) is formed when this carbon carries a methoxyl group.[106]
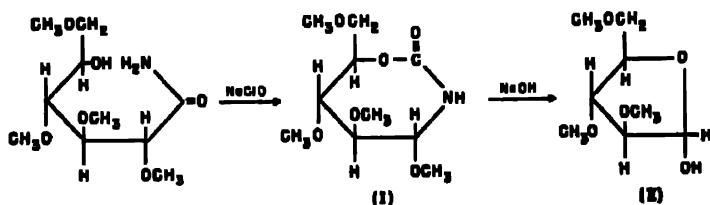
The oxidation of the double bonds of glycals by ozone furnishes an additional method for shortening the carbon chains of the sugars.[107] The oxidation of L-arabinal to L-erythrose provides an example.[108]

[105] R. A. Weerman, Rec. trav. chim., 37, 16 (1917).
[106] W. N. Haworth, S. Peat and J. Whetstone, J. Chem. Soc., 1975 (1938).
[107] E. Fischer, M. Bergmann and H. Schotte, Ber., 53, 509 (1920).
[108] G. E. Felton and W. Freudenberg, J. Am. Chem. Soc., 57, 1637 (1935).

```
HC ──┐
 ‖    │
HC    │                    HC══O
 │    │                     │
HOCH  O      ──O₂→          HOCH
 │    │                     │
HOCH  │                    HOCH
 │    │                     │
H₂C ──┘                    H₂COH
```

L-Arabinal   L-Erythrose
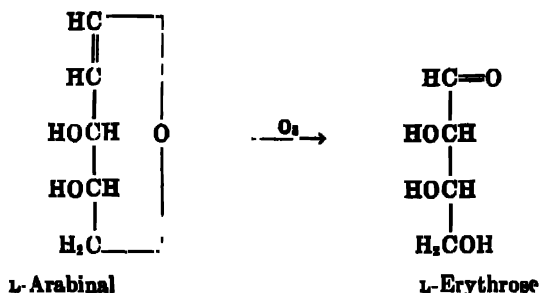
The early work of Kiliani and of Nef (p. 335) demonstrated that the sugars are oxidized by air in alkaline solution with the formation of aldonic and other acids which have fewer carbons in the molecule. Spengler and Pfannenstiel[109] have found that the yields of the aldonic acids with one carbon less than the original sugar can be greatly improved by the use of oxygen rather than air. This oxidation is often of value for elucidating configurational relationships as well as for preparatory purposes. Thus, the formulation of the structure of perseulose as L-galaheptulose is confirmed by its degradation to L-galactonic acid by oxygen and alkali.[110] An extensive study[111] of the reaction reveals that for many sugars the difference between the use of air and oxygen may not be as great as originally thought.

The linkage between adjacent carbons carrying hydroxyl groups is ruptured by lead tetraacetate (p. 329), and sugars with shorter carbon chains are formed. If more than a pair of neighboring hydroxyl groups are available, the oxidation may proceed further so that it is usually desirable to block many of the hydroxyls of the original substance with groups such as isopropylidene, benzylidene, etc. Sorbitol forms 1,2-3,4-diethylidene-sorbitol when treated with paraldehyde and acid. The ethylidene derivative is oxidized by lead tetraacetate to diethylidene-L-xylose which on acid hydrolysis gives L-xylose.[112] The procedure has particular value for the preparation of the lower sugars. The 1,3- and the 2,3-benzylidene-D-arabitol are used for the preparation of D-threose and 1,2-5,6-diisopropylidene-D- or L-mannitol (1) for the important D- or L-glyceraldehyde.[113]

[109] O. Spengler and A Pfannenstiel, Z. Wirtschaftsgruppe Zuckerind., 85, 547 (1935).

[110] N. K Richtmyer, R. M. Hann and C S Hudson, J. Am Chem. Soc., 61, 340 (1939).

[111] H S Isbell, J Research Natl Bur. Standards, 29, 227 (1942)

[112] H. Appel, J. Chem. Soc., 425 (1935).

[113] H. O. L. Fischer and E. Baer, Helv. Chim. Acta, 19, 519 (1936); E. Baer and H. O. L. Fischer, J. Am. Chem Soc., 61, 761 (1939); W. T. Haskins, R. M. Hann and C. S. Hudson, J. Am. Chem. Soc., 65, 1663 (1943).

(I)

L-Glyceraldehyde
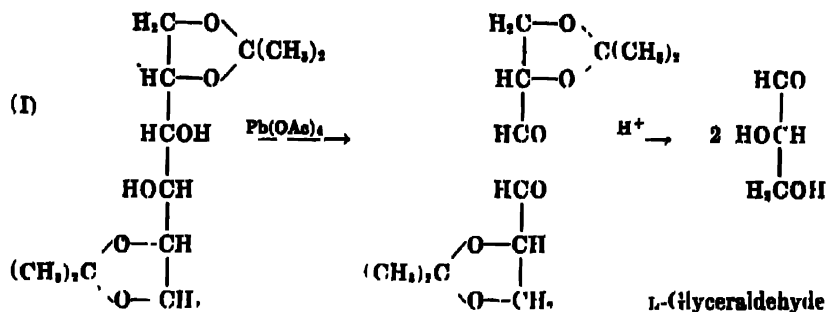
D. Methods for the Synthesis of Sugars with Terminal Methyl Groups (Methyloses).

The synthesis of 6-desoxyglucose (D-glucomethylose, D-isorhamnose) is carried out from 6-bromo-triacetylglucosyl bromide. The dihalide is converted to the methyl 6-bromo-triacetyl-β-D-glucoside by treatment with silver carbonate and methyl alcohol and the bromine is exchanged for a hydrogen atom by reduction with zinc and acetic acid.[114]
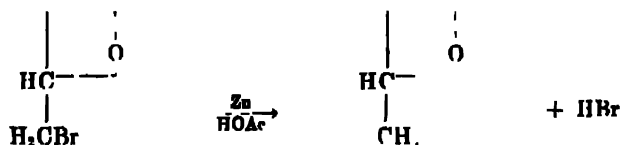
A method of more general application depends on the reduction of iodo derivatives in which the iodine has replaced the hydroxyl of a primary alcoholic group. These are easily obtained by treatment of the tosyl derivatives with sodium iodide in acetone solution (see Tosyl derivatives). The reduction is often carried out by catalytic methods. The 5-desoxy-D-xylose (D-xylomethylose) is synthesized from xylose by this method, and by application of the cyanohydrin synthesis D-gulomethylose is prepared.[115]

D-Xylose $\longrightarrow$ monoisopropylidene-5-tosyl-D-xylose $\xrightarrow{\text{NaI}}$ monoisopropylidene 5-iodo-D xylose $\xrightarrow[\text{NaOH}]{\text{H}_2, \text{Ni}}$ monoisopropylidene-5 desoxy-D xylose $\xrightarrow{\text{H}^+}$ 5-desoxy D-xylose $\xrightarrow{\text{NaCN}}$ 6-desoxy-D gulonic acid $\xrightarrow{\text{Na Hg}}$ 6-desoxy-D-gulose (D-gulo methylose).
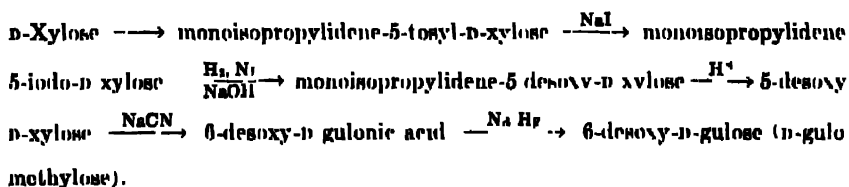
The method has been used by Reichstein and associates[116] for obtaining several 6-desoxy-D-ketohexoses: 6-desoxy-D-fructose, 6-desoxy-D-tagatose and 6-desoxy-L-sorbose.

[114] E. Fischer and K Zach, Ber , 45, 3761 (1912)

[115] P. A. Levene and J. Compton, J Biol. Chem , 111, 325, 335 (1935)

[116] T. Reichstein et al , Helv Chim Acta, 21, 263, 913, 1023 (1938).

The application of the Grignard reagent to aldehyde and carboxyl groups and of diazomethane to *aldehydo* derivatives of the sugars leads to 1-desoxyketoses (see p. 118).

E. **Synthetic Methods Based on Changing the Configuration of Other Sugars.** A number of methods utilized for the synthesis of sugars depend upon changing the configuration of one or more carbon atoms of other sugars. When the configuration of carbon 2 of the aldoses is changed the process is termed an epimerization and this type of change is the most common. The starting materials for these reactions are usually the naturally occurring hexoses and pentoses.

Pyridine and Alkaline Rearrangements. In the presence of alkalies or tertiary amines, the sugars (or the aldonic acids) which differ in the configuration of carbon 2 (2-epimers) establish a pseudo equilibrium. The effect of alkali is more profound for the free sugars than for the acids, and the ketoses are in equilibrium with the 2-epimeric aldoses. The action may proceed still further and involve carbon 3, and under more drastic conditions the entire molecule is degraded (p. 71). The large number of isomers produced from sugars by alkali limits the general application of this method for purposes of synthesis because of the difficulties in the separation of the mixtures and in controlling the reaction.

The reaction brought about by alkalies, called the Lobry de Bruyn-Alberda van Ekenstein transformation, has its major application for obtaining synthetic ketoses which are usually the principal components of the equilibrium. From the mixture produced by the reaction of weak alkali on D-glucose, two ketoses (D-fructose and D-psicose) and two aldoses (D-mannose and D-glucose) have been isolated. Galactose gives analogous products under the same conditions; two ketoses, sorbose and tagatose, and two aldoses, talose and galactose, have been identified.[117] The ketoses lactulose (from lactose) and D-glucoheptulose are easily prepared by this method.[118, 45]

$$
\begin{array}{ccc}
\text{CH}_2\text{OH} & \text{HCO} & \text{COOH} \\
| & | & | \\
\text{C}{=}\text{O} \xrightarrow{\text{sat. Ca(OH)}_2} & \text{HCOH} \xrightarrow{\text{Br}_2} & \text{HCOH} \\
| & | & | \\
\text{HCOH} & \text{HCOH} & \text{HCOH}
\end{array}
$$
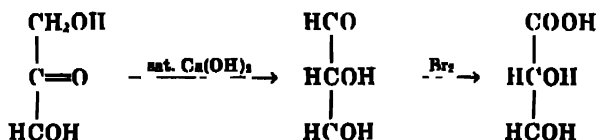
The aldoses in such mixtures may be oxidized by bromine which has no action on the ketoses. The aldonic acid salts formed in this manner are

[117] C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, *16*, 241, 245, 256 (1897); J. U. Nef, *Ann.*, *403*, 342, 362 (1914).

[118] E. M. Montgomery and C. S. Hudson, *J. Am. Chem. Soc.*, *52*, 2101 (1930); W. C. Austin, C. J. Smalley and M. I. Sankstone, *J. Am. Chem. Soc.*, *54*, 1933 (1932)

difficultly soluble in organic solvents and may be separated from the more soluble ketoses.

The isomerizing action of hot pyridine also has considerable value for the preparation of ketoses from aldoses.[119] The two possible D-ketopentoses are prepared by heating the corresponding pentoses with pyridine and fractionally distilling the acetone derivatives of the reaction products. From D-xylose and D-ribose, the amorphous D-xylulose and D-ribulose are obtained.[120]

Omitting consideration of enantiomorphic modifications, it may be pointed out that two of the four possible ketohexoses (D-fructose and L-sorbose) are naturally occurring. The other two, allulose (psicose) and tagatose, have been prepared from the corresponding aldohexoses by the action of pyridine.[121]

The direct action of alkali or pyridine on the sugars is of particular value for the preparation of the ketoses, but the action of pyridine on the aldonic acids is solely an epimerization. The action of hot tertiary amines (particularly aqueous pyridine and quinoline), as well as of alkali, on the aldonic acids and their methylated derivatives results in the establishment of an equilibrium between the two epimeric acids.[122]
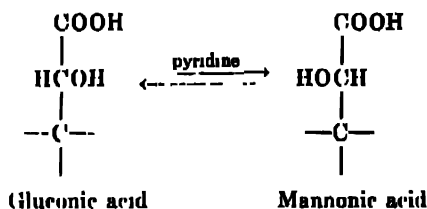
The sugars are prepared by reduction of the corresponding lactones with sodium amalgam. From D-arabonic acid, D-ribose[123] is obtained by this procedure, and from D- and L-galactonic acids, D- and L-talose[124, 125] are prepared.

[119] H. O. L. Fischer, C. Taube and E. Baer, Ber., 60, 479 (1927); S. Danilow, E. Venus-Danilow and P. Schantarowitsch, ibid., 63, 2269 (1930); P. A. Levene and D. W Hill, J. Biol Chem., 102, 563 (1933)

[120] P. A. Levene and R. S. Tipson, J Biol. Chem., 115, 731 (1936); O. Th. Schmidt and K. Heintz, Ann, 515, 77 (1934).

[121] M. Steiger and T. Reichstein, Helv. Chim Acta, 19, 184 (1936); Y. Khouvine, G. Arragon and Y. Tomoda, Bull. soc. chim., [5], 6, 354 (1939).

[122] E. Fischer, Ber., 23, 799 (1890); W. N. Haworth and C. W. Long, J. Chem. Soc., 345 (1929).

[123] M. Steiger, Helv. Chim. Acta, 19, 189 (1936).

[124] W. Bosshard, Helv. Chim Acta, 18, 482 (1935).

[125] C. Glatthaar and T. Reichstein, Helv. Chim. Acta, 21, 3 (1938); O. F. Hedenburg and L H. Cretcher, J Am Chem Soc., 49, 478 (1927)

Glycal Synthesis. By the oxidation of the glycals with perbenzoic acid or hydrogen peroxide (the latter in *tert*-butanol in the presence of OsO_4) two hydroxyl groups are added, and the two corresponding 2-epimeric sugars are produced.[126] The acetylated glycals are obtained by the reduction of the acetylglycosyl bromides (see Glycals).

Tetraacetyl galactosyl bromide

Triacetyl-galactal

Galactal   Galactose   Talose
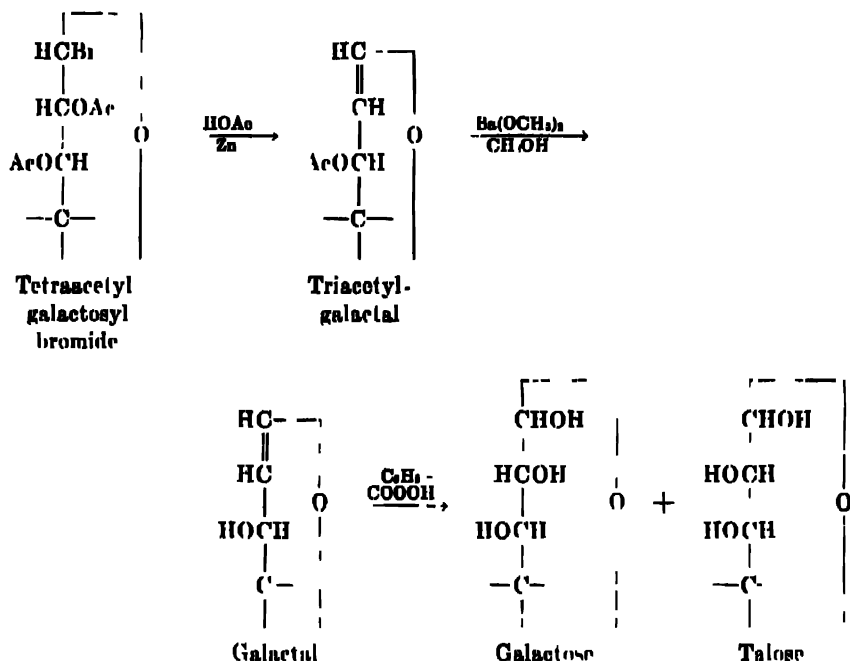
This method often is better than the pyridine rearrangement for bringing about epimerization, and the resulting sugars crystallize more easily than the sugar obtained by the reduction of an aldonic acid. The hexose, D-talose, has been prepared a number of times by the pyridine rearrangement of D-galactonic acid, but the only crystalline material was obtained through the glycal synthesis.[127] From seed crystals prepared in this way, D-talose could then be crystallized from the sirupy product obtained by the reduction of D-talonic acid.[124]

Glycals are also important intermediates for obtaining the 2-desoxy-sugars.[128] The elements of water are added by treatment of the glycal

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[126] M. Bergmann and H. Schotte, *Ber.*, **54**, 440 (1921); R. C. Hockett, A. C. Sapp and S. R. Millman, *J. Am. Chem. Soc.*, **63**, 2051 (1941).

[127] P. A. Levene and R. S. Tipson, *J. Biol Chem.*, **93**, 631 (1931); W. W. Pigman and H. S. Isbell. *J. Research Natl. Bur. Standards*, **19**, 189 (1937).

[128] M. Bergmann, H. Schotte and W. Lechinsky, *Ber.*, **55**, 158 (1922); **56**, 1052 (1923)

with sulfuric acid at low temperature, presumably with the formation of the sulfuric acid ester, and then saponifying with barium carbonate. L-Ribal gives the antipode of the naturally occurring 2-desoxy-D-ribose (2-desoxy-D-arabinose), D-xylal gives 2-desoxy-D-xylose, and D-galactal yields 2-desoxy-D-galactose.[129, 130]

$$
\begin{array}{ccc}
\left[\begin{array}{c} HC \\ \parallel \\ CH \\ | \\ HCOH \\ | \end{array}\right.\left.\begin{array}{c} \\ \\ O \\ \\ \end{array}\right]
&
\xrightarrow{H_2SO_4}
\left[\begin{array}{c} HCOSO_3H \\ | \\ CH_2 \\ | \\ HCOH \\ | \end{array}\right.\left.\begin{array}{c} \\ \\ O \\ \\ \end{array}\right]
&
\xrightarrow{BaCO_3}
\left[\begin{array}{c} HCOH \\ | \\ CH_2 \\ | \\ HCOH \\ | \end{array}\right.\left.\begin{array}{c} \\ \\ O \\ \\ \end{array}\right]
\end{array}
$$

D-Ribal   2 Desoxy-D-ribose
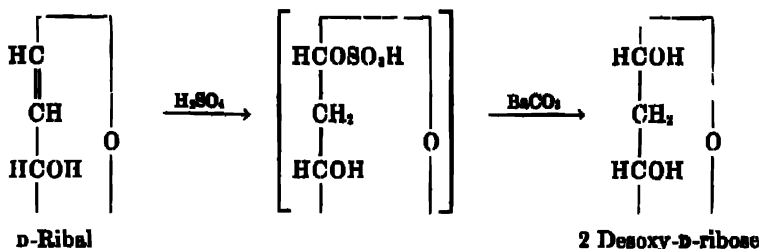
Inversion of All of the Asymmetric Carbons by Transfer of the Aldehyde or Hemiacetal Group. By a transfer of the aldehyde or hemiacetal group from carbon 1 to the terminal atom of the carbon chain, a formal reversal of the configuration of all of the asymmetric centers of the molecule results. The intermediates for this method are the uronic acids, which may be synthesized by a number of methods from the sugars, dibasic acids and alcohols (p. 303). The method[131] depends on the reduction of the aldehyde group of uronic acids; the resulting aldonic acids are converted to lactones and reduced to the sugars. The naturally occurring uronic acids are useful intermediates, particularly the easily prepared D-galacturonic acid which may be reduced to L-galactose (p. 130). Since D-galactose may be converted to D-galacturonic acid by oxidation of the diisopropylidenegalactose with alkaline permanganate, the procedure provides a means for transforming D-galactose to its enantiomorph.[132] The conversion of a sugar into its enantiomorph by this procedure is not the usual result but only occurs for sugars related to inactive dibasic acids. From D-glucose through the intermediate D-glucuronic acid, L-gulose (earlier d-gulose) is obtained. Of the fifteen D- (or L-) aldoses up to and including the hexoses, six are changed to the enantiomorph by the transfer of the aldehyde group, three are unchanged, and six are converted to other sugars. This conversion is

[129] P. A. Levene, L. A. Mikeska and T. Mori, *J. Biol. Chem.*, **85**, 803 (1929).

[130] H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **22**, 397 (1939)

[131] E. Fischer and O. Piloty, *Ber.*, **24**, 521 (1891); H. Thierfelder. *Z. physiol. Chem.*, **15**, 71 (1891)

[132] C. Glatthaar and T. Reichstein *Helv. Chim. Acta*, **20**, 1537 (1937); K. Iwadare and B. Kubota, *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **34**, 183 (1937-38); R. A. Pizzarello and W. Freudenberg, *J. Am. Chem. Soc.*, **61**, 611 (1939), H. S. Isbell, *J. Research Natl Bur. Standards*, **33**, 45 (1944).

generally applicable to all carbohydrate derivatives having unlike terminal groups.

CHO   COOH   COOH
| | |
HCOH   HOCH   HOCH
| | |
HOCH   HCOH   ——Na-Hg——→   HCOH   ——H⁺——→
| or | (alkaline) | heat
HOCH   HCOH   HCOH
| | |
HCOH   HOCH   HOCH
| | |
COOH   CHO   H₂COH

d-Galacturonic   L-Galactonic
    acid   acid

O=C—   O=CH
| | |
HOCH   O   HOCH
| | |
HCOH | ——Na-Hg——→   HCOH
| | (acid) |
HC——   —   HCOH
| |
HOCH   HOCH
| |
H₂COH   H₂COH

Lactone   L-Galactose
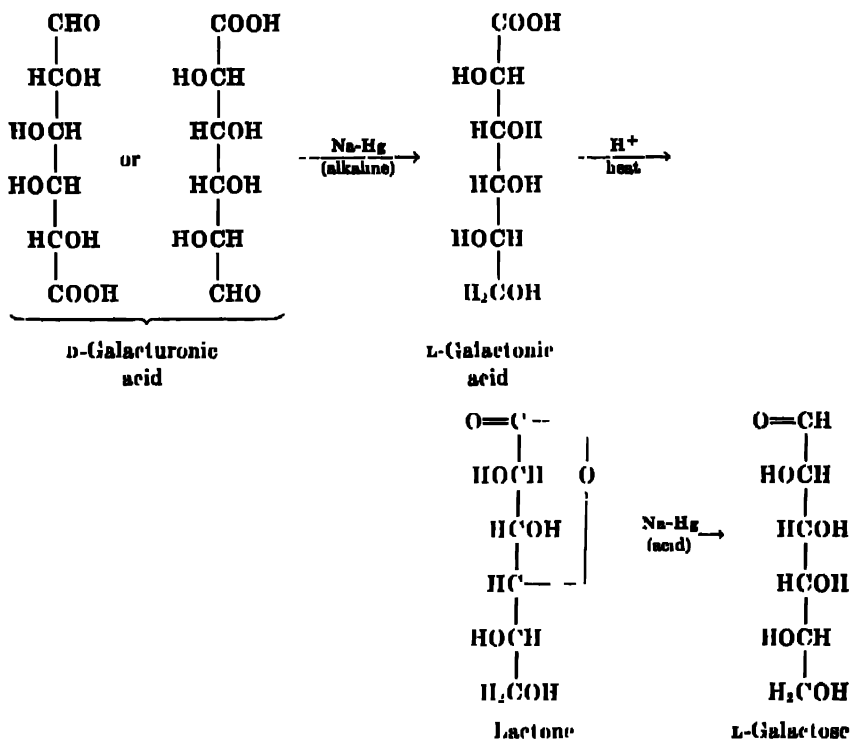
Methods Depending on Walden Inversions. The tosyl esters and other sulfate esters of the sugars often are hydrolyzed with inversion of configuration of the esterified carbons. This process, discussed in more detail under the tosyl esters and anhydrosugars, provides a method for the preparation of sugars and derivatives from the more readily available ones, particularly the naturally occurring sugars. The monoesters are the most important for this purpose since when several groups are hydrolyzed, the concurrent Walden inversions produce many isomers. The hydrolysis takes place first with the formation of an anhydro ring with either or both of the neighboring unsubstituted hydroxyls; the hydrolysis of the anhydro ring is again accompanied by Walden inversion. In most instances, several reaction products are formed, of which one is a derivative of the original sugar.

Several other methods result in inversions of configuration probably as the result of Walden inversions taking place during the decomposition of intermediate compounds. Thus, the action of AlCl₃ and PCl₅ on octaacetylcellobiose produces a derivative of the new disaccharide celtrobiose. The

process results in a change from the D-glucose to the D-altrose configuration. Although the latter method has value for the production of D-altrose, the principal utility of these procedures is for the production of the rarer disaccharides and more details are given in the section on disaccharide synthesis.

F. Preparation of Ketoses by Biochemical Oxidation of Alcohols. Of considerable importance for the preparation of the ketose sugars is the oxidation of the sugar alcohols by bacteria. It was first observed that the occurrence of L-sorbose in the juice of the mountain ash (*Sorbus aucuparia*) is due to the bacterial oxidation of the precursor, D-sorbitol. The bacteria responsible were isolated and their oxidizing action on a series of polyhydroxyalcohols was extensively studied by Bertrand.[133] The active organism was shown to be identical with *Acetobacter xylinum* previously isolated from vinegar by Adrian Brown. According to the generalization of Bertrand, the oxidation of alcohols by *A. xylinum* is favored by a *cis* relationship of two secondary hydroxyl groups adjacent to a primary alcoholic group. The secondary hydroxyl adjacent to the primary hydroxyl is then oxidized to a ketone group by the organism. Glycerol is oxidized to dihydroxyacetone.

$$(HO)H_2C\text{-}CH(OH)\text{-}CH_2(OH) \rightarrow (HO)H_2C\text{-}CO\text{-}CH_2(OH)$$
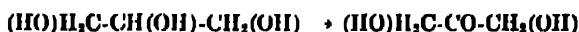
Another organism, *Acetobacter suboxydans*, found originally in beer, often gives better results than *A. xylinum*. It has been found particularly effective for manufacturing L-sorbose from D-sorbitol and has made this

		CH$_2$OH		CH$_2$OH
		HOCH		C=O
		HOCH		HOCH
D-Glucose	electrolytic reduction →	HCOH	bacteria →	HCOH
		HOCH		HOCH
		CH$_2$OH		CH$_2$OH
		Sorbitol		L-Sorbose
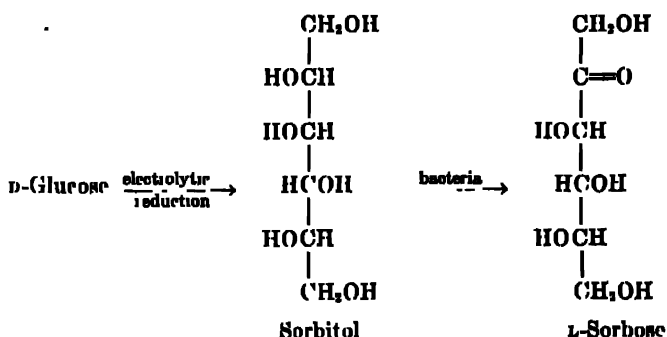
sugar available at low cost.[134] The organism gives L-erythrulose by oxidation of *meso*-erythritol and dihydroxyacetone by oxidation of glycerol.[135]

[133] G. Bertrand, *Ann. chim.*, [8] 3, 181 (1904).

[134] E. I. Fulmer, J. W. Dunning, J. F. Guymon and L. A. Underkofler, *J. Am. Chem. Soc.*, 58, 1012 (1936); P. A. Wells *et al.*, *Ind. Eng. Chem.* 31, 1518 (1939); U. S Patent 2,121,533, June 21, 1938; A J. Kluyver and F J de Leeuw, *Tijdschr Vergelijk Geneeskunde*, 10, 170 (1924).

[135] See: R. L. Whistler and L. A. Underkofler, *J. Am. Chem. Soc.*, 60, 2507 (1938); K. R. Butlin, *J. Soc. Chem. Ind.*, 57, T468 (1938).

The presence of a small amount of glucose (0.05 per cent) as well as aeration and agitation promotes the oxidation of perseitol to perseulose and seems to provide optimal conditions for the oxidation of other alcohols.[71] The action of *A. suboxydans* is more specific than the *A. xylinum* as demonstrated by studies of the action of the former on a number of sugar alcohols.[137] Although the same groupings are necessary, there is a marked difference between the actions of *A. suboxydans* on enantiomorphs. D-Arabitol is oxidized to D-xylulose but L-arabitol is not attacked. Oxidation of L-rhamnitol does not take place but does for L-fucitol. The action of the organism is not limited to the sugar series since the closely analogous *meso*-inositol, a cyclic alcohol, yields a pentahydroxycyclohexanone called inosose.[138] The biological oxidation of α-glucoheptitol to L-glucoheptulose provides a method of passing from D-glucose to L-glucose. The alcohol is obtained by the reduction of the product of the cyanohydrin synthesis from D-glucose, and the L-glucoheptulose yields L-gluconic acid (and other acids) when subjected to the action of oxygen in alkaline solution.[139a]

The chemical oxidation of sugar alcohols to ketoses may be successful when only one unsubstituted hydroxyl group is present. Thus, 6-benzoyl-1,3-2,4-diethylidene-D-sorbitol is oxidized to the corresponding L-sorbose derivative by the action of chromic acid dissolved in glacial acetic acid.[139b]

G. Aldose to Ketose Conversion Utilizing the Osones. A method of considerable historical interest is the transformation of the aldoses to the ketoses through the osazones and osones.[140] In his classical work which led to the synthesis of the isomeric sugars, Fischer utilized this procedure. Although better preparative methods are now available, the method still is important for demonstrating structural relationships. The osazones are transformed to the osones by treatment with concentrated hydrochloric acid or aldehydes (p. 406). The reduction of the osone to the ketose is brought about by the action of zinc and acetic acid.

HC=NNHC₆H₅   HC=O   CH₂OH
   | | |

$$\begin{array}{ccccc}
\text{HC=NNHC}_6\text{H}_5 & & \text{HC=O} & & \text{CH}_2\text{OH} \\
| & & | & & | \\
\text{C=NNHC}_6\text{H}_5 & \xrightarrow{\text{HCl}} & \text{C=O} & \xrightarrow[\text{HOAc}]{\text{Zn}} & \text{C=O} \\
| & & | & & | \\
\text{—C—} & & \text{—C—} & & \text{—C—} \\
| & & | & & | \\
\text{Glucosazone} & & \text{Glucosone} & & \text{Fructose}
\end{array}$$
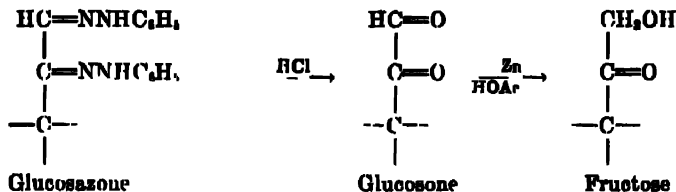
[136] E. B. Tilden, *J. Bact.*, **37**, 629 (1939).

[137] R. M. Hann, E. B. Tilden and C. S. Hudson, *J. Am. Chem. Soc.*, **60**, 1201 (1938).

[138] A. J. Kluyver and A. Boezaardt, *Rec. trav. chim.*, **58**, 956 (1939); T. Posternak, *Helv. Chim. Acta*, **24**, 1045 (1941)

[139a] W. D. Maclay, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 1066 (1942); N. K. Richtmyer and C. S. Hudson, *ibid.*, **64**, 1609 (1942)

[139b] W. R. Sullivan, *J. Am. Chem. Soc.*, **67**, 837 (1945).

[140] E. Fischer, *Ber.*, **22**, 87 (1889).

3. The Identification and the Quantitative Determination of Carbohydrates[141]

A. Qualitative Identification

a. SEPARATION OF SUGAR MIXTURES

Many methods have been employed for the identification of sugars. When only a single sugar is present in the material undergoing examination, the methods customary to organic chemistry may be used. Thus, derivatives may be prepared, and the properties can be compared with those of known materials. The optical rotation of the unknown and of its derivatives provides one of the best properties for the identification. Mixtures are much more difficult to analyze. Distillation as a means of fractionation is limited because of the ease of decomposition and of the low volatility of sugars and derivatives. However, the methyl ethers and the propionic esters can be distilled without decomposition, and they are used for the separation of sugar mixtures[142] (see under the Discussion of the Structures of the Polysaccharides). The most widely applicable method for the separation is the fractional crystallization of the sugar mixture or of a derivative of the mixture.

The chromatographic separation of azoyl derivatives of sugars has been studied.[143] The p-phenylazobenzoyl ("azoyl") esters of the sugars are red colored products that are prepared by the reaction of the acid chloride with the sugar in pyridine solution. The azoyl esters, in solution, may be

$$\text{Glucose} + p\text{-}C_6H_4\text{---}N\text{=}N\text{-} \quad C_6H_4\text{---}COCl \xrightarrow{\text{pyridine}}$$

$$C_6H_7O_5(C_6H_4\text{---}N\text{=}N\text{---}C_6H_4CO)_5$$
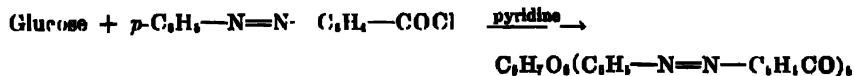
adsorbed on a column of an appropriate inert material such as alumina, silica, silicic acid or magnesium silicate, and a colored band is obtained. If a solvent is passed through the column, the band will move down the column. The adsorbed azoates of a mixture of sugars may be separated by

[141] The present discussion was abstracted from: C. A. Browne and F. W. Zerban, "Sugar Analysis": John Wiley, New York (1941). F. J Bates and Associates, "Polarimetry, Saccharimetry and the Sugars," Circular 440 of the Natl. Bur. Standards, Government Printing Office, Washington, D. C. (1942). The reader is referred to these and other excellent discussions of the subject for more complete details and references. References to particular methods are given only when they cannot be found in the above works.

[142] C. D. Hurd and associates, J. Am Chem. Soc., 63, 2656, 2657, 2659 (1941); C. D. Hurd, D. T. Englis, W. A. Bonner and M. A. Rogers, ibid., 66, 2015 (1944). For the application of the methyl ethers to the analytical separation of sugars see: C D Hurd and S M Cantor, ibid., 60, 2677 (1938). See also Chapters XII to XV for products obtained by the hydrolysis of polysaccharides

[143] W. S Reich, Biochem J, 33, 1000 (1939), G. H Coleman and C M McCloskey, J. Am. Chem Soc, 65, 1588 (1943), G H Coleman, D E Rees, R. L Sundberg and C M. McCloskey, ibid., 67, 381 (1945).

passing solvent through the column. The bands representing the least easily adsorbed substances pass down the column first, and under good conditions separate distinct bands are obtained for each component of the mixture. The bands may be separated mechanically by breaking the column; the azoates are recovered by elution from the adsorbent by extraction with a solvent. The method has been successfully applied for the separation of mixtures such as: glucose and fructose; glucose and cellobiose; arabinose, glucose, trehalose and cellobiose. Adsorption analyses have been applied directly to sugar mixtures.[144] Since the sugars are colorless, the passage of adsorption bands out of the column is indicated by measurements of the density or refractive index of the eluate. Streak reagents may be used to indicate the positions of bands of adsorbed material. The process has been applied to the separation of the products obtained by the action of enzymes on starch.

b. Color Reactions

The presence of "carbohydrates" is indicated by the development of colors when the unknown is treated with strong sulfuric acid and an appropriate phenol. α-Naphthol is one of the most commonly used phenols; others are resorcinol, orcinol, phloroglucinol and cresol. The test employing α-naphthol is known as the Molisch test for carbohydrates. The colored substances probably are condensation products between the phenols and furfural, hydroxymethylfurfural and similar products formed from the sugars by the action of the acids. This type of reaction can be used for the quantitative estimation of carbohydrates (see below). The reaction is given by the simple sugars, the oligosaccharides and by many polysaccharides.

Strong sulfuric and hydrochloric acids convert carbohydrates to dark colored substances which probably are condensation products of furfural, hydroxymethylfurfural, etc.

The colors produced from ketoses, pentoses and uronic acids in the presence of phenols and acids as well as other reagents often are enough different from those formed from aldohexoses so that they may be used for the classification of unknown materials. The ketoses, pentoses and uronic acids usually form colored products under conditions milder than those required for the aldohexoses. Tauber's benzidine test for pentoses and uronic acids involves the heating of benzidine (NH_2-C_6H_4-C_6H_4-NH_2) in glacial acetic acid with the sugar. A cherry red color forms in the presence

[144] A. Tiselius, Kolloid-Z., 105, 101 (1943); A. Tiselius and L. Hahn, ibid., 105, 177 (1943); B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., J. Am. Chem. Soc., 68, 1449 (1946). Simple qualitative and quantitative micro methods have been described: S. M. Partridge, Nature 158, 270 (1946); A. E. Flood, E. L. Hirst and J. K. N. Jones, ibid., 160, 86 (1947).

of pentoses and glucuronic acid, whereas hexoses give a yellow to brown color. Phloroglucinol gives a violet-red color with pentoses and uronic acids in the presence of hydrochloric acid. Orcinol may be used to distinguish between pentoses and uronic acids. The Seliwanoff test for ketoses is carried out by heating the unknown with hydrochloric acid and resorcinol. A fiery-red color develops if a ketose is present.

A particularly important color reaction is the Raybin diazouracil test for sucrose (see under Sucrose). An alkaline solution of diazouracil turns green in the presence of sucrose. The only interfering substances are raffinose, gentianose and stachyose.

The reduction of metallic salts provides a convenient test for "reducing" sugars. In alkaline solution, the sugars reduce the salts of copper, silver, mercury and other metals to the metal or to a suboxide. The well-known Fehling and Tollens solutions are of this character. The sugar and the products resulting from isomerization in alkaline solution (see Chapter II) are oxidized to the corresponding acids.

$$2 \; Cu(OH)_2 \;+\; \underset{\displaystyle -C-}{\overset{\displaystyle HCO}{|}} \quad \longrightarrow \quad \underset{\displaystyle -C-}{\overset{\displaystyle HOCO}{|}} \;+\; Cu_2O \;+\; H_2O$$
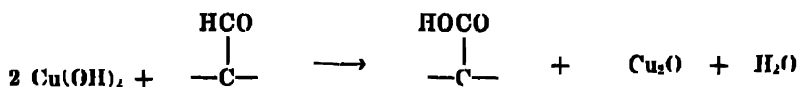
The formation of the metal or oxide is taken as evidence for the presence of reducing sugars. Similar reactions are given by many substances other than carbohydrates. The application of this test to the quantitative determination of sugars is described in the next section.

Strong alkalies cause solutions of reducing sugars to turn dark brown, particularly when the solutions are hot. The nature of the products is unknown.

Reducing sugars reduce nitrophenols to deeply colored derivatives. Picric acid, $C_6H_2OH(NO_2)_3$, is transformed to the deep red salt of picramic acid, $C_6H_2(NO_2)_2(NH_2)OH$. For o-dinitrobenzene, the test is so sensitive that 6 parts per 1,000,000 of reducing sugars may be detected.

Methylene blue solutions are decolorized by alkaline solutions of reducing sugars. Safranine changes from a red to a yellow color under similar conditions.

c. Derivatives

The reaction products of the reducing sugars and aromatic hydrazines are very useful derivatives for identification purposes. One mole of hydrazine may react to give the sugar hydrazone, or two residues may be introduced to give the osazones. Phenylhydrazine is the most common hydrazine used for this purpose, but other hydrazines are used. The choice

of hydrazine depends upon the sugar present since the products differ greatly in their case of isolation. For example, mannose phenylhydrazone is difficultly soluble, whereas the glucose phenylhydrazone is quite soluble.
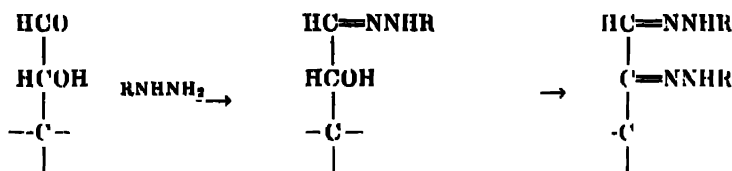
The osazones are much less soluble than the hydrazones. However, it should be noted that three sugars (e.g., glucose, mannose and fructose) give the same osazone because of the loss of asymmetry at carbon atom 2. (For further details of this reaction, the reader is referred to the discussion of nitrogenous derivatives, Chapter IX).

The benzimidazoles of the aldonic acids have been suggested for the identification of sugars and acids.[145] The benzimidazoles are made by oxidation of the sugars to the aldonic acids, and subsequent condensation of the aldonic acids with o-phenylenediamine.

Aldonic acid   Aldobenzimidazole
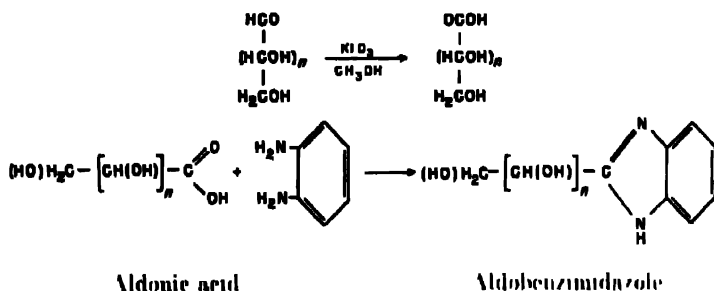
The separation of small quantities of the aldobenzimidazoles is facilitated by the formation of the insoluble copper salt from which the copper may be removed by exposure to hydrogen sulfide. The melting points and optical rotations of the benzimidazoles and of the corresponding hydrochlorides differ sufficiently for the different sugars so that the identification is assured. Fructose under the conditions outlined above is likely to be oxidized with the production of small quantities of D-arabo-benzimidazole. Characteristic derivatives of hexuronic and saccharic acids also are obtained by condensation with o-phenylenediamine.

Derivatives of particular value for the identification of many important sugars are mentioned in Chapters III, IX and X under the description of the individual sugars.

[145] See: S. Moore and K. P. Link, J. Biol. Chem., 133, 293 (1940); R. J. Dimler and K. P. Link, ibid., 150, 345 (1943).

B. Quantitative Determination. Many of the qualitative tests may be applied to the quantitative determination of sugars. The color developed in the presence of acids and phenols or the amount of metal or metallic oxide formed by the reduction of the salts of heavy metals by the sugars can be measured. In some cases, difficultly soluble derivatives such as the osazones can be weighed directly. Because of the absence of a stoichiometric relation or because of the appreciable solubility of derivatives, most of these methods are not completely satisfactory. The discovery of a true stoichiometric reaction or of derivatives insoluble in the presence of other sugars would be a valuable contribution to the analysis of carbohydrates.

a. Optical Rotation

When sugars or their derivatives are reasonably pure, and in particular are free of optically active impurities, the measurement of the optical rotation provides the most convenient method for their identification and analysis. This method of "direct polarization" finds wide application in the analysis of raw and purified cane and beet sugar. The specific rotation of a sugar in solution is given by:

$$[\alpha]_D^{2t} = \frac{100\alpha}{l \times c}$$

(α = observed optical rotation; l = length of tube in decimeters; c = weight of sugar (grams) in 100 ml. of solution at 20°C.). When the specific rotation is known, the concentration, c, may be calculated from:

$$c = \frac{100\alpha}{l \times [\alpha]_D^{20}}$$

Usually the specific rotation varies somewhat with the concentration (c), and this effect must receive consideration.

The method is very easily applied when a saccharimeter is used for the measurement of the rotation. In this procedure, the weight of impure sugar which is taken for the analysis is the same as the amount of pure sugar which will read $100°S$ under the same conditions. The observed optical rotation gives directly the percentage of sugar in the sample. Thus, a reading of $90°S$ would mean that the original material contained 90 per cent of the sugar. The weight of a sugar which will read $100°S$ on a saccharimeter when made up to 100 ml. at 20°C. and read in a 2-dm. tube is known as the normal weight. For sucrose, the normal weight is 26.00 g.

Mixtures of several sugars are more difficult to analyze by optical rotation methods, but sometimes the analysis is possible if the rotations of the components vary in a different manner when the solvent, the acidity or the

temperature is changed. The change in solvent may be brought about by the addition of salts such as borax which markedly affect the rotations. If the specific rotations of the two components are known under two sets of conditions, the solution of two simultaneous equations will give the relative percentages of components x and y:

Condition 1: $x [\alpha_x] + y [\alpha_y] = 100 [\alpha_{obs}]$.

Condition 2: $x [\alpha_x'] + y [\alpha_y'] = 100 [\alpha_{obs}']$.

One of the most important sugar mixtures which can be analyzed by the optical rotatory method is the mixture of sucrose and its hydrolysis products, glucose and fructose. The process of hydrolysis of sucrose into glucose and fructose is known as inversion because of the change of the sign of rotation which takes place during the hydrolysis. Mixtures of this type are found in invert sirups, honey, etc. The polarimetric method for this purpose is based on the measurement of the optical rotatory power of the original material and of the completely hydrolyzed product.

$$C_{12}H_{22}O_{11} \quad + \quad H_2O \quad \longrightarrow \quad C_6H_{12}O_6 \quad + \quad C_6H_{12}O_6$$

Sucrose   Glucose + Fructose

Invert sugar
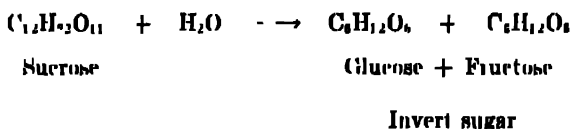
From the known rotations of sucrose and of its hydrolysis products, the quantity of sucrose in the original mixture may be calculated. This method originally was devised by Biot (1842), but it was greatly improved by Clerget and bears the name of the Clerget method. Acids have been employed as the catalysts for the hydrolysis reaction. However, the instability of fructose under acid conditions, and the marked influence of acids and salts on its optical rotation are likely to lead to erroneous results unless the conditions are carefully controlled. The inversion by yeast invertase gives more accurate results.

The results are calculated from the formula:

$$S = \frac{100(P - P')}{133 - 0.5(t - 20)}$$

where P and P' are the observed optical rotations before and after acid hydrolysis and t is the temperature (°C.) at which the rotations are measured. The constant 133 is the Clerget constant. The percentage of sucrose is given by S. The method must be carried out under carefully standardized conditions. (For further details and discussion, the reader is referred to the previously mentioned works on the subject.)

b. Reducing Sugar Methods

Oxidation by Metallic Salts in Alkaline Solution. The principal chemical methods for quantitatively determining the sugars make use of the reducing action of sugars on alkaline solutions of the salts of certain metals. Although many metallic salts, including those of copper, silver, mercury, and bismuth undergo this type of reaction, copper has been employed by far the most extensively in sugar analysis. The reaction might be visualized as the case of an aldehyde or ketone being oxidized by withdrawal of oxygen from the base formed by the action of the alkali upon the salt. The reduced base is precipitated either as the free metal or as the suboxide. However, the process is complicated by the extensive action of the alkali

$$RCHO + Ag_2O \rightarrow 2Ag + RCOOH$$

$$RCHO + 2CuO \rightarrow Cu_2O + RCOOH$$
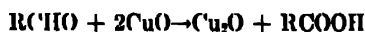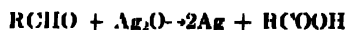
upon the sugar and by the presence of alcoholic groups which also are oxidizable. By careful control of the conditions, a complete breakdown of the carbon chain may be achieved in accordance with the following equation:

$$C_6H_{12}O_6 + 9Ag_2O \rightarrow 18Ag + 3 HOOC \cdot COOH + 3H_2O$$

<div align="center">Oxalic acid</div>
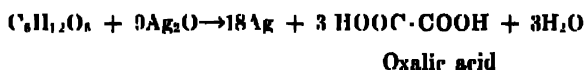
Under these conditions the amount of glucose may be estimated from the weight of the reduced silver. However, the reaction seldom proceeds stoichiometrically. It has been shown previously (see Chapter II) that sugars with free aldehyde and ketone groups quickly undergo change even in weakly alkaline solution. Glucose, fructose and mannose undergo a mutual interconversion until equilibrium is established. This interconversion is explained by the formation of an enol form. Upon prolonged action the double bond may descend farther along the chain. Strong alkalinity produces more deeply seated changes forming saccharinic acids and their lactones. In the presence of cupric salts in alkaline solutions, the enediols are oxidized at the expense of the cupric ions which are reduced and precipitated as insoluble cuprous oxide. The carbon chain of the sugar is ruptured with the formation of acids with shorter chains. Since the enediol bond of a hexose at the time the molecule is ruptured may be either at the 1,3; the 2,3; or the 3,4 position and since the hydroxyls may have altered their positions, a large number of acids is produced.

Under such circumstances it is amazing that the reaction has quantitative value. But it has been found that, although the products are many and variable, it is possible to standardize the conditions so that the amount of cuprous oxide may be used as a measure of the quantity of sugar.

Copper solutions became important for purpose of sugar analysis after Trommer (1841) used alkaline copper sulfate to distinguish between grape sugar (glucose) and cane sugar (sucrose). In 1844, Barreswil reported the important discovery that the addition of potassium tartrate to alkaline copper sulfate solution greatly increases the stability. The reaction of the tartrate with the copper salt is still not clearly understood, but it is generally assumed that complex salts are formed. Cupric tartrate is precipitated when a solution of copper sulfate is added to a chemically equivalent amount of sodium tartrate in solution. If a second equivalent of sodium hydroxide is added, the precipitated cupric tartrate dissolves. Since the resulting solution is neutral to litmus, the whole cupric tartrate residue acts as an ion to neutralize the alkali. That the copper is a constituent of the anion is shown by electrolysis of the solution; under these conditions the copper migrates to the anode. The reagent used for sugar analysis must have more than one equivalent of alkali for one of the tartrate because the sugar enol is formed only in alkaline solution.

Citrates, oxalates, salicylates, carbonates, glycerol, and cane sugar stabilize alkaline solution of cupric salts in a manner similar to the action of tartrates. Some of these, citrates in particular, have been used in the preparation of copper solutions for sugar analysis.

The copper method was further improved in 1848 by Fehling, who worked out analytical details of the alkaline copper method essentially as they now stand. Fehling gave as stoichiometrical equivalents: 5 molecules of copper to one molecule of glucose. But apparently he did not realize that the amount of copper which is reduced varies with experimental conditions and is quantitative only within a narrow range of concentrations and of reaction times. The ratio 1:5 was employed subsequently until Soxhlet in 1878 showed that the ratio varies with the degree of excess of copper present during the reaction. Soxhlet's method was also an improvement in that he kept the copper solution and the alkaline tartrate solution in separate containers; the solutions were mixed at the time of analysis. The composition of the Fehling (Soxhlet) reagents is as follows:

Fehling solution A: 34.639 g. crystalline copper sulfate ($CuSO_4 \cdot 5H_2O$) made up to 500 ml. with water.

Fehling solution B: 173 g. Rochelle salt and 50 g. NaOH made up to 500 ml. with water.

Since the copper reduction method has become used so generally for sugar analysis, numerous modifications have been described which are based on the same fundamental principles but which differ in analytical details. Fehling solution is rather unstable. Hence, efforts have been made

to improve its stability. Many organic products other than sugars cause either a precipitation of cuprous oxide or prevent its precipitation even if sugars are present. Consequently, other copper solutions are frequently employed, especially in biological analysis. Copper sulfate or acetate usually is used as the source of the cupric ion. Potassium hydroxide has been substituted for sodium hydroxide in the method of Allihn and in its modifications. Citrates or carbonates have been used instead of sodium or potassium hydroxide to produce reagents having less alkalinity as for the solutions of Benedict and of Soldaini. Among other copper solutions recommended for testing sugars, copper ammonium tartrate and ammoniacal copper sulfate may be mentioned. But with all the numerous modifications the Fehling-Soxhlet solution is the most widely used of the copper solutions. No other has been found to equal it for general usefulness in sugar analysis although others may be more suitable under specific circumstances.

The amount of copper which is reduced by various sugars has been found to vary according to the alkalinity, the temperature, the time of heating, the sugar concentration, the nature of the sugar, the type of the tartrate (d, l or meso), the amount of contact with air, etc. Fehling solution approximates the degree of alkalinity which has been found to give the largest deposit of cuprous oxide. Two of the most important variables are the temperature and the time of heating. Initially, the reduction is very rapid as the temperature is raised to 75°C. The rapid phase is followed by a slow secondary reduction which continues for a long time. However, the rate of reduction is very slow at the later time periods. In most methods the solution is allowed to boil until a point is reached at which a small variation in the time will exert only a negligible influence on the results. Because of the arbitrary establishment of the conditions and the absence of a stoichiometric relation between the quantity of sugar and the cuprous oxide formed, close adherence to the conditions described for the various methods is required. Under standardized conditions, the amount of cuprous oxide is proportional to the initial quantity of sugar. For many methods, tables have been published which relate the quantity of sugar and the amount of cuprous oxide or copper. The multiplicity of tables arises from the fact that many investigators have confined their work to one single sugar for one individual set of conditions. The early tendency was to devise a particular method for each sugar under examination. This procedure requires different reagents and procedures for each sugar and renders impossible the interpretation of copper equivalents for mixtures of sugars. This difficulty led to the establishment of unified procedures for which the same reagents and procedure are used regardless of the nature of the sugar. Empirical copper equivalents have been determined for the sugars of common occurrence and for the most frequently occurring sugar mixtures.

Among the unified methods are those of Munson and Walker (the most common method in the United States), of Quisumbing and Thomas, of Bertrand, of Brown, Morris and Millar, of Lane and Eynon and of Scales (modified).

After the establishment of standard conditions for the reduction, considerable variation is possible in the method for determining the cuprous oxide. It may be weighed directly or ignited to cupric oxide. It may be further reduced to metallic copper by hydrogen, by alcohol vapor or by electrolysis in nitric acid solution. In other procedures, the cuprous oxide is dissolved after filtration and is determined volumetrically by use of ferric salts and permanganate, iodine and thiosulfate, thiocyanate and silver salts, dichromate and ferrous salts or the cyanide method. In the cyanide method, the excess of cupric ion is determined. Several processes have also been worked out for the determination of the extent of the reduction without filtration of the cuprous oxide. Titration may be made of the cuprous ion or of the excess cupric ion. Ferric ion oxidation of the dissolved cuprous oxide is employed in the Bertrand method. The Scales, the Shaffer-Hartmann and the Shaffer-Somogyi methods employ iodometric determination of the cuprous ion in the presence of citrates which form complex ions with cupric ions. The Folin-Wu method and its modification according to Benedict require measurement of the color produced by cuprous salts and a tungstic acid reagent.

Instead of measuring the copper reduced by a given amount of sugar, the copper solution may be titrated directly by the addition of sugar to the boiling copper solution. The end point is distinguished by the discharge of the blue color (methods of Violette and of Pavy), by spot tests with ferrocyanide (Soxhlet) or by the internal indicator methylene blue (Lane and Eynon). Other indicators have been suggested; in the case of very dark molasses, the end point preferably is determined electrometrically. Main's "pot method" was devised because it is difficult to standardize the time of heating and the rate of ebullition. The temperature is regulated by a boiling water-bath, and the reduction is carried out in test tubes provided with floats, variable amounts of sugar being added to constant amounts of copper reagent. The same principle is used, but constant amounts of sugar solution are added to variable quantities of copper reagent in the method of Reischauer and Kruis.

Although the reduction of cupric salts in alkaline solution is common to all aldoses and ketoses (as well as aldehydes and ketones), conditions may be established for which a preferential oxidation of monosaccharides takes place. In the Barfoed method, copper acetate in neutral or slightly acid solution oxidizes monosaccharides but affects disaccharides such as maltose only to a minor degree. The Steinhoff method for the selective

determination of glucose, maltose and dextrins in mixtures depends on the determination of glucose by the Barfoed reagent, the sum of dextrose and disaccharides (maltose) by use of Fehling solution, and the total sugar after complete acid hydrolysis.

Descriptions of the procedures followed in the various methods and tables relating the sugar quantity to the amount of cuprous oxide or copper are given in the standard works on analysis.

Oxidation with Potassium Ferricyanide. A number of important methods are based on the oxidation of sugars by ferricyanide ion in alkaline solution. The method is open to the same objections as the copper reduction methods, namely, the lack of a stoichiometric reaction and the dependence of the method on arbitrarily chosen conditions. The ferricyanide may be used to titrate the sugar solution directly by the use of picric acid or of methylene blue as an indicator. Or, the reduced ferrocyanide may be precipitated as the zinc salt, and the excess ferricyanide determined iodometrically. The Hagedorn-Jensen method and the Hanes modification utilize the latter procedure. Extensive application of the ferricyanide method has been made in the determination of the diastatic power of amylase preparations and in blood analysis.

$$4 \ K_3Fe(CN)_6 + 4 \ KOH \rightarrow 4 \ K_4Fe(CN)_6 + 2 \ H_2O + O_2$$

$$2 \ H_3Fe(CN)_6 + 2 \ HI \leftrightarrows 2 \ H_4Fe(CN)_6 + I_2$$

$$2K_4Fe(CN)_6 + 3 \ ZnSO_4 \rightarrow K_2Zn_3[Fe(CN)_6]_2 + 3 \ K_2SO_4$$

c. Colorimetric Procedures Using Orcinol or Carbazole

The formation of colored products by the reaction of sugars and phenols in the presence of strong acids has been mentioned previously as a qualitative test for carbohydrates. Carbazole (I) may be used instead of a phenol.
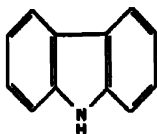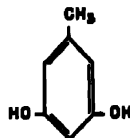
(I)   (II)

By use of a colorimeter (or preferably a spectrophotometer) and the rigorous standardization of the conditions of the analysis, it is possible to employ this type of reaction for the qualitative estimation and the quantitative determination of minute quantities of carbohydrates in biological products. Methods employing orcinol (3,5-dihydroxytoluene, II) and carbazole have been described in detail.[148]

[148] M. Sørensen and G Haugaard, *Biochem. Z.*, *260*, 247 (1933); S. Gurin and D. B Hood, *J. Biol Chem*, *139*, 775 (1941); *151*, 211 (1939)

The absorption curves for the different sugars after treatment with orcinol or carbazole and strong sulfuric acid differ considerably. Hence, the shape of the absorption curve frequently is of value in the identification of an unknown sugar even in the presence of amino acids and other materials.

Quantitative evaluation is possible by colorimetric comparison of a sample of the unknown with a standard solution of the same sugar.

d. SPECIAL METHODS

Determination of Aldoses by Hypoiodite. Romijn (1897) first showed that aldoses are quantitatively oxidized by iodine in weakly alkaline solution under carefully controlled conditions. Ketoses and non-reducing sugars are only slightly attacked. Equations illustrating the reaction are given below.

The iodine and alkali form hypoiodite and iodide:

$$I_2 + 2\ NaOH \rightarrow NaIO + NaI + H_2O$$

Part of the hypoiodite is converted into iodate and iodide, the amount depending on the concentration, the time and the temperature:

$$3\ NaIO \rightarrow NaIO_3 + 2\ NaI$$

The hypoiodite reacts with the aldose:

$$RCHO + NaIO + NaOH \rightarrow RCOONa + NaI + H_2O$$

Since sodium iodate cannot oxidize the sugar in alkaline solution some active iodine is lost as far as the sugar oxidation is concerned. If the entire quantities of alkali and iodine are admitted simultaneously, much iodine is transformed to iodate and a deficiency may result for the sugar oxidation. If iodine is present in too great an excess, over-oxidation can occur, and the alcoholic groups are slowly oxidized to carboxyl or carbonyl groups.

Although some iodine may be lost by the side reaction, this iodine is measured along with the excess when the solution is acidified and titrated with thiosulfate.

$$NaIO + NaI + H_2SO_4 \rightarrow I_2 + Na_2SO_4 + H_2O$$

$$NaIO_3 + 5\ NaI + 3H_2SO_4 \rightarrow 3I_2 + 3Na_2SO_4 + 3H_2O$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2\ NaI + Na_2S_4O_6$$

Slater and Acree found that the iodine consumption can be confirmed by titrating with alkali the free acid left after the completion of the thiosulfate titration.

$$HCl + NaOH \rightarrow NaCl + H_2O$$

$$\text{Aldonic lactone} + H_2O \rightleftharpoons RCOOH$$

$$RCOOH + NaOH \rightarrow RCOONa + H_2O$$
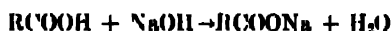
Although the stoichiometrical nature of this reaction is an advantage over the empirical nature of the copper reductions, this procedure does not have as great a versatility of application, and it also must be used under carefully controlled conditions. Alcohol, glycerol, mannitol, formic acid, lactic acid, dextrin, amino acids, and many other substances take up iodine. Hence, the method cannot be applied directly to impure sugar products of unknown composition. Two well-known modifications of the original Romijn method are the Willstätter-Schudel and the Klein-Acree methods.

Determination of Pentoses and Pentosans. Pentose sugars and pentosans may be quantitatively estimated by conversion into furfural by distillation with hydrochloric acid. The amount of furfural is determined gravimetrically after precipitation with phlorglucinol, barbituric acid or thiobarbituric acid, or volumetrically by titration with bromine or phenylhydrazine. Approximately theoretical yields of furfural are obtained if the furfural is removed rapidly from the reaction mixture by steam distillation.

$$C_5H_{10}O_5 \longrightarrow C_5H_4O_2 + 3H_2O$$

Pentose   Furfural

Hexoses yield hydroxymethylfurfural, and methyloses yield methylfurfural. These substances are not produced in quantitative yields, and they interfere with the furfural determination. (More details of this reaction are given on pages 69 and 308.)

Determination of Sugars as Hydrazones and Osazones. The solubility of the different hydrazones and osazones or of similar derivatives in the presence of impurities has prevented their general employment for the quantitative separation of the sugars. In certain cases, however, where they are characterized by great insolubility, they may be used for fairly accurate quantitative determinations. Arabinose may be determined by precipitating it with diphenylhydrazine; mannose with phenylhydrazine; and fructose with methylphenylhydrazine. Some osazones may be determined volumetrically. Glucosazone, for example, is reported to be reduced stoichiometrically by titanium trichloride to isoglucosamine.

Fermentation Methods. The selective fermentation of sugars by micro-organisms is utilized for the qualitative and quantitative determination of sugar mixtures. Ordinary yeasts ferment glucose at alkalinities up to pH 8 although maltose is only slowly fermented above pH 7.2. This difference

TABLE II

Fermentative Characteristics of Some Microorganisms[17]

Organism	D,L-Glyceric aldehyde	D-Arabinose	L-Arabinose	D-Ribose	L-Ribose	D-Lyxose	D-Xylose	L-Rhamnose	D-Glucose	D-Mannose	D-Galactose
B. megatherium	A	0	A	A	0	0	A	0	A	AG	0
Serratia marcescens	AG	A	A	A	0	A	0	0	A	A	A
Es. coli	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
A. aerogenes	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Bact. friedlanderi	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Proteus vulgaris	A	A	0	0	A	A	A	0	A	A	0
S. aertrycke	AG	A	AG	AG	A	AG	AG	AG	AG	AG	AG
S. enteritidis	A	A	AG	AG	A	A	AG	AG	AG	AG	A
S. cholerae-suis	A	A	0	AG	0	AG	AG	AG	AG	AG	AG
S. paratyphi	A	0	AG	AG	A	A	A	AG	AG	AG	AG
S. Schottmulleri	A	A	AG	AG	AG	AG	AG	AG	AG	AG	AG
E. typhi	A	0	0	A	0	0	0	0	A	A	0
E. dysenteriae, Flexner	A	0	A	A	0	0	0	0	A	A	0
E. dysenteriae, Sonne	A	A	A	A	A	A	A	A	A	A	A
Sarcina lutea	A	0	0	0	0	A	0	0	0	0	0
Staph. aureus	A	0	A	A	A	0	A	0	A	0	0
Staph. albus	A	0	A	A	A	A	A	0	A	A	A
Sac. cerevisiae	0	0	0	0	0	0	0	0	AG	AG	0
Torula cremoris	0	0	0	0	0	0	0	0	AG	AG	0

A, acid formation observed.

G, gas formation observed.

0, no reaction.

has been made the basis of the Somogyi method for the determination of glucose, maltose and dextrins in products such as are obtained by the hydrolysis of starch. The determination of the reducing power of a sample before fermentation, after fermentation at pH 7.5, and after fermentation at pH 5.0 provides a method for the selective determination of maltose,

[17] L. Sternfeld and F. Saunders, *J. Am. Chem. Soc*, 59, 2053 (1937) See also: C. M. McCloskey and J R. Porter, *Proc. Soc. Exptl. Biol. Med.*, 60, 260 (1945).

glucose and unfermentable (dextrin) material. Instead of reducing sugar determinations, measurements of the alcohol concentration may be used to measure the degree of fermentation. Mixtures such as are obtained by the hydrolysis of starch also may be analyzed by the use of a yeast which will not ferment maltose, and one which will act on this sugar.[146]

TABLE III

Fermentative Characteristics of Some Microorganisms[147]

Organism	Sorbitol	D Gluconic acid	Dulcitol	D Galactonic acid	Mucic Acid	Inositol	Erythritol	Methyl α D-glucoside	Glucosamine	D Gluconose
B megatherium	0	A	0	0	0	0	0	0	0	0
Serratia marrescens	A	A	0	0	0	A	A	0	A	0
Es coli	AG	AG	AG	AG	A	0	0	0	AG	0
A aerogenes	AG	AG	0	0	AG	AG	0	AG	AG	0
Bact friedlanderi	AG	AG	AG	AG	AG	AG	0	AG	AG	0
Proteus vulgaris	0	AG	0	0	0	0	0	AG	0	A
S. aertrycke	AG	AG	AG	AG	AG	0	0	0	A	0
S enteritidis	AG	A	AG	A	A	0	0	0	A	0
S cholerae suis	AG	AG	0	0	0	0	0	0	A	0
S paratyphi	AG	AG	A	0	0	0	0	0	A	0
S Schottmulleri	AG	AG	AG	AG	A	AG	0	0	A	0
E typhi	A	A	0	0	0	0	0	0	0	0
E dysenteriae, Flexner	A	A	0	A	0	0	0	A	0	0
E dysenteriae, Sonne	0	A	0	A	A	0	0	0	0	0
Sarcina lutea	0	0	0	0	0	0	0	0	0	0
Staph aureus	0	A	0	0	0	0	0	0	A	0
Staph albus	A	A	A	0	A	A	A	A	A	A
Sac cerevisiae	0	0	0	0	0	0	0	A	0	0
Torula cremoris	0	0	0	0	0	0	0	0	A	A

A, acid formation observed

G, gas formation observed.

0, no reaction.

Wise and Appling[149] determine galactose in the presence of mannose, glucose, fructose, xylose, arabinose and glucuronic acid by use of a yeast (*Saccharomyces carlsbergensis*) which ferments galactose and one (*S. bayanus*) which does not Both yeasts ferment mannose, glucose and fructose

[146] See for example A S Schultz, R. A Fisher, L Atkin and C N Frey, *Ind Eng Chem, Anal Ed*, 15, 496 (1943)

[149] L E Wise and J W Appling, *Ind Eng Chem, Anal Ed*, 16, 28 (1944)

but have no action on xylose, arabinose and glucuronic acid. Mixtures of this type are obtained by the hydrolysis of plant gums.

The accompanying Tables II and III illustrate the marked specific action of bacteria and yeasts on sugars and derivatives.[110] By the proper application of microorganisms, it is possible to provide evidence for the presence of a given sugar in an unknown mixture. Thus as shown in the accompanying tables, an evidence of fermentation by *Torula cremoris* combined with an absence of fermentation by ordinary yeasts (*Sac. cerevisiae*) would be indicative of the presence of glucosamine. In turn, the fermentation characteristics of a microorganism is used for its identification. The latter use provides the main application for many of the rarer sugars.

ESTERS

In the carbohydrate group as a whole, including polysaccharides, the dominant functional group is the hydroxyl group, particularly if the hemiacetal group is considered as a hydroxyl. The sugar alcohols (polyols), glycosides and polysaccharides have primary and secondary alcoholic groups, and the sugars, these groups and also carbonyl or hemiacetal groups.

$$
\begin{array}{ccc}
 & R' & OR'' \\
 & | & | \\
R\text{—}CH_2OH & R\text{—}CHOH & R'\text{—}COH \\
\text{Primary alcohol} & \text{Secondary alcohol} & \text{Hemiacetal}
\end{array}
$$
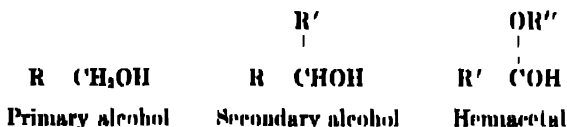
One of the most common reactions of hydroxyl groups is that of esterification. The present chapter covers the ester derivatives of carbohydrates other than polysaccharides. Since the esters of the sugars have received the most study, the discussion will be centered about them.

The ester derivatives of polysaccharides are of considerable industrial importance, e.g., cellulose acetates and nitrates. Those of the sugars have not been commercialized to any great extent, with the possible exception of sucrose octaacetate. An important series of surface-active materials are provided by polyols and their anhydro derivatives partially esterified with long-chain fatty acids. For the latter derivatives, increased solubility in water is provided by reaction with ethylene oxide.

A few esters of the sugars occur in natural products. Vaccinin (mono benzoylglucose) is found in the juice of blueberries; populin (salicin 6-benzoate) occurs in *Populus* species. Phosphate esters of hexoses, trioses and hydroxy acids act as intermediates in the biological synthesis of polysaccharides, ethyl alcohol (alcoholic fermentation) and lactic acid (glycolysis).

Esterification is accomplished by reaction of the carbohydrate with an acyl halide or an acid anhydride and catalyst. The catalyst may be an acid (H_2SO_4, HCl, $ZnCl_2$, etc.) or a nitrogenous base such as pyridine. Acids are likely to hydrolyze glycosidic bonds if present, whereas bases may cause rearrangements if reducing sugars are used. In common with other organic esters, these derivatives are hydrolyzed by both acids and alkalies, with alkalies being particularly effective. The fully substituted organic esters are soluble in organic solvents, particularly well in the chlorinated hydrocarbons. If fully esterified, the products usually are easily crystallized and obtained in high yield.

The ease of reactivity of the OH groups is usually in the order: Hemiacetal OH, primary alcohol, secondary alcohol. The presence of ring structures, however, has a great influence on the reactivity. For the sugars, cyclic esters (usually pyranoses) are the principal products obtained on esterification, but sometimes small amounts of acyclic esters (derivatives of the *aldehydo*-form) are among the reaction products.

ACYL DERIVATIVES

1. Acetyl Derivatives

A. Cyclic Acetates. The acetyl derivatives of the sugars have been extensively employed as intermediates in sugar synthesis and for the isolation and identification of the sugars. Their value for these purposes arises from their ease of preparation and crystallization and because the acetyl groups are easily removed. As early as 1860, Berthelot[1] obtained a sirupy ester by reacting glucose and glacial acetic acid. Liebermann[2] introduced the use of anhydrous sodium acetate and acetic anhydride, and Franchimont[3] obtained from glucose, sodium acetate and acetic anhydride a crystalline ester which was probably β-pentaacetylglucose. The use of zinc chloride as a catalyst in place of sodium acetate gave the α-pentaacetylglucose[3] although, because of difficulties in analysis, the two pentaacetates were not recognized at the time as being isomers. These two catalysts are still

β-Glucose pentaacetate   α-Glucose   α Glucose pentaacetate
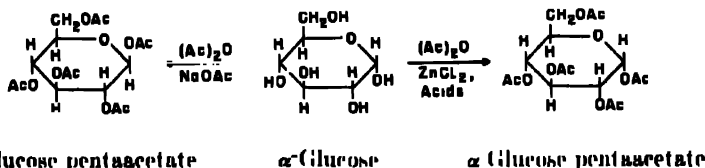
used extensively for acetylation, but pyridine, sulfuric acid and perchloric acid have advantages as catalysts in many instances.[4] The catalytic efficiencies of perchloric acid, phosphoric acid and zinc chloride are related to their relative proton affinities (relative acidities) in the acetylating medium.[5]

For the acetylation of β-naphthol, the reaction has been shown to be subject to both acidic and basic catalysis.[6] The rate of acetylation is slow-

[1] M. Berthelot, *Ann. chim. phys.*, [3] *60*, 93 (1860).

[2] C. Liebermann and O. Hörmann, *Ber.*, *11*, 1618 (1878); A. P. N. Franchimont, *Ber.*, *12*, 1940 (1879).

[3] E. Erwig and W. Koenigs, *Ber.*, *22*, 1464 (1889).

[4] A. Verley and Fr. Bölsing, *Ber.*, *34*, 3354 (1901); R. Behrend and P. Roth, *Ann.*, *331*, 362 (1904); A. P. N. Franchimont, *Compt. rend*, *92*, 1053 (1881); D. Krüger and A. Roman, *Ber.*, *69*, 1830 (1936).

[5] D. Krüger, *Nitrocellulose*, *9*, 175 (1938).

[6] J. B. Conant and G. E. Bramann, *J. Am. Chem. Soc.*, *50*, 2305 (1928)

est between pH 1 and 3 ·(in glacial acetic acid, acetic anhydride) and increases at both higher and lower acidities. These results may apply to the acetylation of carbohydrates.

The acetylation of the nonreducing sugars and other derivatives which consist of a single modification can be carried out by almost any method which does not affect glycosidic linkages, but the acetylation of the reducing sugars is complicated by the existence of several ring modifications. For this reason it is necessary to select a method which will give the desired product. The isomer obtained depends upon the catalyst used in the acetylation and upon the temperature. The following general scheme[7] illustrates the effect of these factors on the acetylation of glucose. At low temperatures (0°C.) the equilibriums represented by reactions I and II are only slowly established and the acetylation reactions III or IV take place without isomerization. By the use of pyridine and a low tempera-

(IV)

β-D-Glucose $\xrightarrow{\text{H}_2\text{SO}_4, \text{ZnCl}_2, \text{pyridine (cold)}}_{\text{Sodium acetate (hot)}}$ Pentaacetyl-β-D-glucose

(I) $\Updownarrow$ $\Updownarrow \uparrow$ $\begin{smallmatrix}\text{ZnCl}_2,\text{H}_2\text{SO}_4,\\ \text{or HCl}\end{smallmatrix}$ (II)

α-D-Glucose $\xrightarrow{\text{Acids, ZnCl}_2(\text{hot or cold})}_{\text{Pyridine (cold)}}$ Pentaacetyl-α-D-glucose

(III)
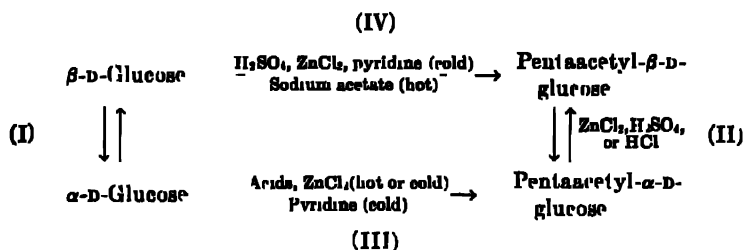
ture, the α-aldohexose yields the α-pentaacetate, and the β-aldohexose yields the β-pentaacetate. At higher temperatures, in the presence of acid catalysts, isomerization between the acetates takes place, and the products obtained depend upon the position of the equilibrium represented by the reaction II. In the case of glucose, the equilibrium mixture of the pentaacetates consists of 90 per cent of the alpha and 10 per cent of the beta pentaacetylglucose.[8] For many sugars the alpha acetates predominate in the equilibrium mixture and consequently the use of acid catalysts, such as zinc chloride, and a relatively high temperature (20° to 110°C.) produces the alpha acetate from either the alpha or beta sugar. With sodium acetate as a catalyst at a high temperature, the equilibrium (I) between the alpha and beta sugars is established, whereas the equilibrium (II) between the acetates is not. Since the beta sugar is acetylated more rapidly than the alpha, the principal product is then the beta acetyl sugar. The diagram also illustrates how the alpha acetates may be prepared from the beta acetates. For this purpose, a mixture of sulfuric acid, acetic acid and acetic anhydride has certain advantages over zinc chloride.[9] This reagent also

[7] C. S. Hudson, *J. Ind. Eng. Chem.*, **8**, 380 (1916).

[8] C. L. Jungius, *Z. physik. Chem.*, **52**, 101 (1905).

[9] E. Montgomery and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 2463 (1934).

brings about the transformation of acetylated glycosides to the corresponding acetyl-α-aldoses. In contradiction to the general belief that the reaction is only catalyzed by acid catalysts, solid sodium hydroxide in an inert solvent also catalyzes the transformation of the beta to the alpha acetyl sugars.[10] The acetylation reaction usually gives high yields, but particularly for reducing sugars, many isomers are formed. The reaction has been used as a means for the determination of hydroxyl groups in organic compounds.[11]

The application of ketene ($CH_2=CO$) to the acetylation of carbohydrates has been investigated.[12] Although methyl 6-trityl-α-glucoside and 1,2-isopropylidene-glucose are completely acetylated by ketene in hot dioxane or acetone, only three of the four hydroxyl groups of methyl α-glucoside are acetylated by this treatment and glucose itself is not affected.

<div align="center">

TABLE 1

Properties of Pentaacetylgalactoses[a]

</div>

Substance	m. p. (°C)	$[\alpha]_D$
α Pentaacetylgalactopyranose	96	106 7
β- "	142	25
β-Pentaacetylgalactofuranose	98	-11 6
α "	87	61 2
α-Pentaacetylgalactoheptanose	128	11 0
β "	101	78 3 (18°C)
Aldehydo-pentaacetylgalactose	121	25 (26°C)

[a] Rotations in chloroform

More complete esterification can be brought about by the presence of sulfuric acid in the reaction mixture.

The number and types of crystalline isomers of acetyl sugars frequently is greater than for the parent sugars. Thus, seven isomeric pentaacetylgalactoses are known; these include three ring types (pyranoses, furanoses, heptanoses) and the open-chain *aldehydo*-pentaacetylgalactose. The properties of the seven pentaacetylgalactoses are given in Table I.

B. Acyclic Sugar Acetates (Aldehydo Derivatives). The open-chain forms of the sugars are presumed to be the intermediates in certain reactions, such as mutarotation, but, as far as is known, crystalline sugars always exist in one of the ring forms. In aqueous solutions, there is considerable evidence that appreciable amounts of the aldehyde or aldehydrol form exist in equilibrated solutions of sugars such as ribose and fructose

[10] M. L. Wolfrom and D. Husted, *J. Am. Chem. Soc.*, 59, 364 (1937).

[11] See M. Freed and A. M. Wynne, *Ind. Eng. Chem.*, *Anal. Ed.*, 8, 278 (1936). B. E. Christensen and R. A. Clarke, *ibid.*, 17, 265 (1945)

[12] C. D. Hurd, S. M. Cantor and A. S. Roe, *J. Am. Chem. Soc.*, 61, 426 (1939).

(see p. 67). Amorphous D-glucose pentamethyl ether (II), prepared[13] by the methylation of glucose diethyl mercaptal (I) and removal of the mercaptal groups, has an acyclic structure.

$$
\begin{array}{cccc}
\text{HCO} & \text{HC(SC}_2\text{H}_5)_2 & \text{HC(SC}_2\text{H}_5)_2 & \text{HCO} \\
| & | & | & | \\
\text{HCOH} \xrightarrow[\text{HCl}]{\text{C}_2\text{H}_5\text{SH}} & \text{HCOH} \dashrightarrow & \text{HCOCH}_3 \longrightarrow & \text{HCOCH}_3 \\
| & | & | & | \\
\text{HOCH} & \text{HOCH} & \text{CH}_3\text{OCH} & \text{CH}_3\text{OCH} \\
| & | & | & | \\
& \text{(I)} & & \text{(II)}
\end{array}
$$
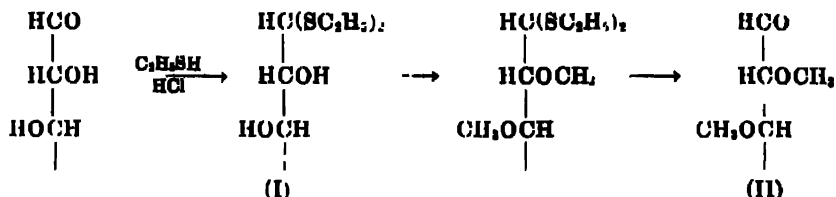
The acetyl and benzoyl derivatives of the free-aldehyde and ketone forms of the sugars are well characterized crystalline substances and have been extensively investigated by Wolfrom and by Brigl.[14] The sugar mercaptals (III), oximes (IV) and semicarbazones have been applied to their preparation.

$$
\begin{array}{c}
\text{RSH} \\
+ \\
\text{Glucose}
\end{array}
\xrightarrow[\text{HCl}]{\text{conc}}
\begin{array}{c}
\text{HC(SR)}_2 \\
| \\
\text{HCOH} \\
| \\
\text{HOCH} \\
| \\
\text{HCOH} \\
| \\
\text{HCOH} \\
| \\
\text{H}_2\text{COH} \\
\text{(III)}
\end{array}
\xrightarrow[\text{(Ac)}_2\text{O}]{\text{NaOAc}}
\begin{array}{c}
\text{HC(SR)}_2 \\
| \\
\text{HCOAc} \\
| \\
\text{AcOCH} \\
| \\
\text{HCOAc} \\
| \\
\text{HCOAc} \\
| \\
\text{H}_2\text{COAc}
\end{array}
\xrightarrow[\text{CdCO}_3]{\text{HgCl}_2}
\begin{array}{c}
\text{HC}{=}\text{O} \\
| \\
\text{HCOAc} \\
| \\
\text{AcOCH} \\
| \\
\text{HCOAc} \\
| \\
\text{HCOAc} \\
| \\
\text{H}_2\text{COAc} \\
\textbf{(V)} \\
\uparrow \\
\text{HNO}_2
\end{array}
$$

$$
\begin{array}{c}
\text{NH}_2\text{OH·HCl} \\
+ \\
\text{Glucose}
\end{array}
\xrightarrow{\text{KOAc}}
\begin{array}{c}
\text{HC}{=}\text{NOH} \\
| \\
\text{HCOH} \\
| \\
\text{HOCH} \\
| \\
\text{(IV)}
\end{array}
\xrightarrow{\text{(Ac)}_2\text{O}}
\begin{array}{c}
\text{HC}{=}\text{NOAc} \\
| \\
\text{HCOAc} \\
| \\
\text{AcOCH} \\
|
\end{array}
\xrightarrow{\text{(COOH)}_2}
\begin{array}{c}
\text{HC}{=}\text{NOH} \\
| \\
\text{HCOAc} \\
| \\
\text{AcOCH} \\
|
\end{array}
$$
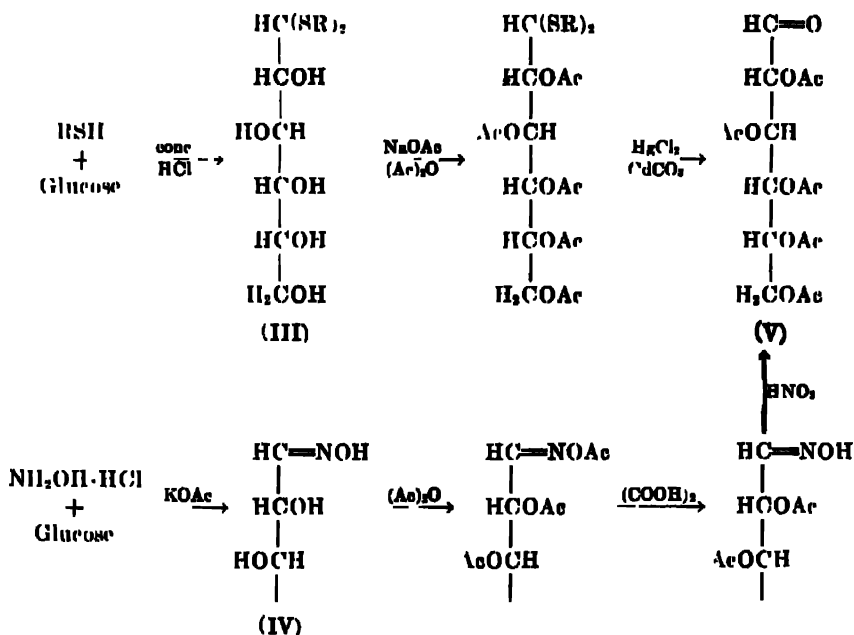
Thiol esters may be reduced to give aldehydes:

$$
\underset{\text{R---C---SR'}}{\overset{\text{O}}{\|}} \xrightarrow[\text{Ni}]{\text{H}_2} \underset{\text{R---C---H}}{\overset{\text{O}}{\|}}
$$
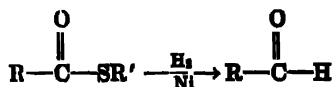
[13] P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **69**, 175 (1926).

[14] M. L. Wolfrom, *J. Am. Chem. Soc.*, **51**, 2188 (1929); P. Brigl and H. Mühlschlegel, *Ber.*, **63**, 1551 (1930).

A small yield of *aldehydo*-D-ribose tetraacetate has been obtained by the reduction of ethyl thiol-D-ribonate tetraacetate.[15]

The sugar mercaptals are perhaps the best intermediates except that the reaction conditions may be too severe for some sugars, e.g., the disaccharides which may be hydrolyzed.[16] The use of the oximes is complicated by the formation of acetylated nitriles during the acetylation process.

The products prepared in this way are true aldehydes or ketones and exhibit the typical reactions of these compounds. They give positive tests with Schiff's reagent, whereas the sugars give a positive test only under carefully controlled conditions. Catalytic reduction of an *aldehydo* acetyl sugar produces the corresponding alcohol but each *keto* acetyl sugar yields two alcohols since a new asymmetric center is produced.[17] Oxidation of *aldehydo* acetyl sugars by bromine produces the corresponding acetylated aldonic acids.[18]

Of particular interest for distinguishing between the cyclic and the free-aldehyde forms of the acetyl (and benzoyl) sugars is the reaction with ethyl mercaptan in the presence of zinc chloride as a catalyst.[19] The penta-acetylglucopyranoses (I) and furanoses (the ring forms) lose an acetyl group in the formation of a thioglucoside (II) but *aldehydo*-pentaacetyl-glucose (III) loses none and forms the mercaptal (IV).

HC=O	HC(SR)₂	HCOAc	HC·SR
(CHOAc)₄	(CHOAc)₄	HCOAc	HCOAc
CH₂OAc	CH₂OAc	AcOCH O	AcOCH O + HOAc
		HCOAc	HCOAc
		HC———	HC———
		H₂COAc	H₂COAc
(III)	(IV)	(I)	(II)

$$HC=O \xrightarrow[ZnCl_2]{RSH} HC(SR)_2$$
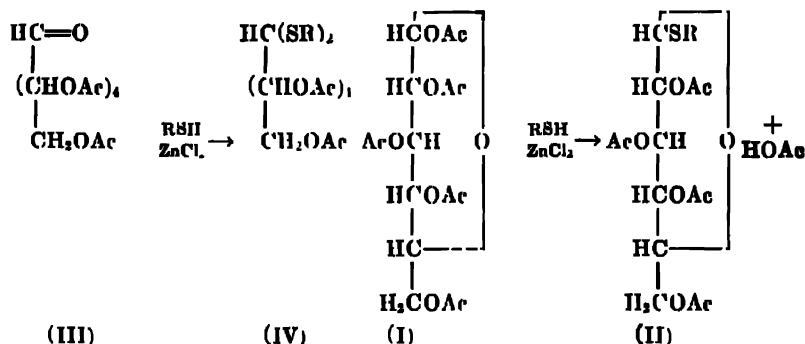
The acetylglyconic aldehydes mutarotate in aqueous and alcoholic solutions and form crystalline hydrates and alcoholates which are believed to be aldehydrol or hemiacetal derivatives.[20]

[15] M. L. Wolfrom and J. V. Karabinos, *J. Am Chem Soc.*, **68**, 724 (1946).

[16] M. L. Wolfrom, L. W Georges and S. Soltzberg, *J. Am. Chem. Soc.*, **56**, 1794 (1934).

[17] E Pacsu and F V. Rich, *J Am Chem Soc*, **55**, 3018 (1933).

[18] R. T Major and E. W. Cook, *J. Am. Chem Soc*, **58**, 2474 (1936).

[19] M. L. Wolfrom and A. Thompson, *J Am Chem. Soc*, **56**, 880, 1804 (1934); P. Brigl and R. Schinle, *Ber*, **66**, 325 (1933)

[20] M. L. Wolfrom, *J. Am. Chem, Soc.*, **53**, 2275 (1931), M. L. Wolfrom and W. M. Morgan, *ibid.*, **54**, 3390 (1932).

H
|
HOCOH   HC=O   ROCOH
| |
(HCOAc)₄ ←ROH— (HCOAc)₄ ROH (HCOAc)₄
| | |
H₂COAc   H₂COAc   H₂COAc
Aldehydrol   Free-Aldehyde   Hemiacetal
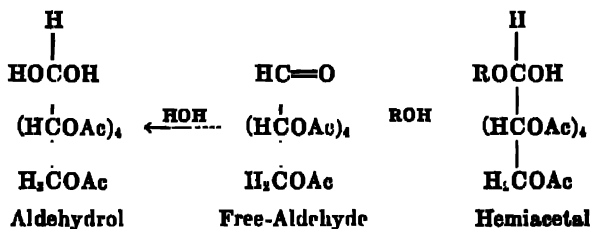
The free-aldehyde form of acetylated hexoses also reacts[21] with acyl

halides to give 1-halogeno-hexaacetates, $CH_2OAc—(HCOAc)_4—\overset{OAc}{\underset{X}{CH}}$

(X = I, Br, Cl), and upon acetylation with sodium acetate and acetic anhydride yields heptaacetates, $CH_2OAc—(HCOAc)_4—CH(OAc)_2$.

Open-chain acetates of the sugars are found occasionally among the products obtained by the usual methods of acetylation of the sugars. Thus, Pacsu and Rich demonstrated that the long known "alpha" penta-acetylfructose of Hudson and Brauns has a free ketonic group. By the acetylation of certain heptoses with sodium acetate and acetic anhydride, the open-chain acetyl derivatives are formed directly.[22]

Phosphorus pentachloride reacts with ordinary aldehydes to produce the 1,1-dihalides. It reacts similarly with aldehydo-pentaacetylgalactose to give the 1,1-dichloride, but in ether solution phosphoric acid esters are obtained.[23]

A reaction of the acetylated straight-chain compounds of interest for synthetic purposes is that with diazomethane.[24] By this reaction a carbon atom is added and a 1-desoxyketose derivative formed (see also p. 118.)

CH₃
|
HC=O   C=O
| |
(CHOAc)₃ ⁻CH₃N₂→ (CHOAc)₃
| |
CH₂OAc   CH₂OAc

aldehydo-Tetraacetyl-   1-Desoxy-tetraacetyl-
arabinose   keto fructose
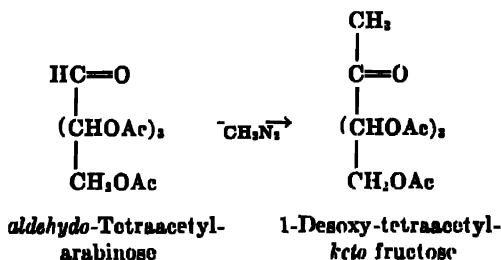
C. Acetates with a Heptanose Ring. By a special series of reactions a pair of cyclic pentaacetylgalactoses with seven-atom rings (heptanose

[21] M. L. Wolfrom, J. Am. Chem. Soc., 57, 2498 (1935).
[22] E. Montgomery and C. S. Hudson, J. Am Chem. Soc., 56, 2463 (1934).
[23] M. L. Wolfrom and D. I. Weisblat, J. Am Chem. Soc., 62, 1149 (1940).
[24] M. L. Wolfrom, D. I. Weisblat, W. H. Zophy and S. W. Waisbrot, J. Am. Chem. Soc., 63, 201 (1941).

or septanose rings) have been synthesized by Micheel and Suckfüll.[25] The method of synthesis follows:

Galactose → 6-tritylgalactose → 6-trityl-1,2-3,4-diisopropylidenegalactose

$\xrightarrow{\text{NaI}}$ 6-iodo-diisopropylidenegalactose $\xrightarrow{\text{H}^+}$ 6-iodogalactose $\xrightarrow[\text{HCl}]{\text{EtSH}}$

6-iodogalactose diethyl mercaptal → 6 iodo-tetraacetylgalactose diethyl

mercaptal $\xrightarrow[\overline{\text{HgCl}_2}]{\text{CdCO}_3}$ aldehydo-2,3,4,5 tetraacetylgalactose $\xrightarrow{\text{pyridine}}$

2,3,4,5 tetraacetylgalactoheptanose $\xrightarrow{(\text{Ac})_2\text{O}}$ α and β-1,2,3,1,5-pentaacetylgalactoheptanoses.
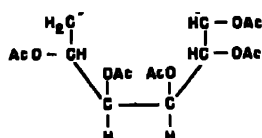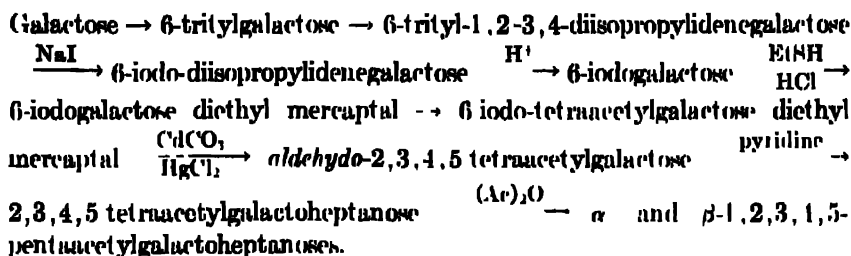
Pentaacetylgalactoheptanose (crystalline)

The heptanose structure was confirmed[26] by conversion of the pentaacetate to a methyl tetramethylgalactoheptanoside which upon oxidation with nitric acid gives tetramethylmucic acid, COOH- (CHOCH₃)₄ COOH·

Pentaacetylgalactoheptanose $\xrightarrow[\text{CHCl}_3]{\text{TiCl}_4}$ 1-chloro tetraacetylgalacto-

heptanose $\xrightarrow[\text{Ag}_2\text{CO}_3]{\text{CH}_4\text{OH}}$ Methyl tetraacetylgalactoheptanoside $\xrightarrow{\text{NaOCH}}$

Methyl galactoheptanoside $\xrightarrow[\text{Ag}_2\text{O}]{\text{CH}_3\text{I}}$ Methyl tetramethylgalactohep-

tanoside $\xrightarrow{\text{HNO}_3}$ 2,3,4,5-tetramethylmucic acid.
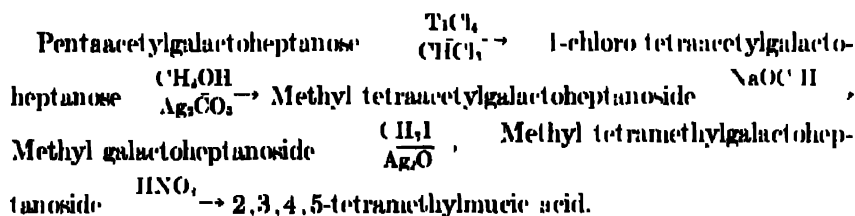
D. Derivatives of Orthoacetic Acid.[27] The three ring types and the open-chain form do not exhaust the types of isomerism found in the acetylated sugars. The first representative of the new type was prepared by Fischer, Bergmann and Rabe,[28] who by reacting triacetylrhamnosyl bromide with methyl alcohol in the presence of silver carbonate, obtained a compound with the same analysis as an acetylated methyl rhamnoside but which exhibited the unique property of having one acetyl group resistant to alkaline hydrolysis. A similar derivative of mannose was then reported by

[25] F. Micheel and F Suckfull Ann, 502, 85 (1933).

[26] F. Micheel and F Suckfull. Ann, 507, 138 (1933)

[27] H W Post, "The Chemistry of the Aliphatic Orthoesters", p 106, Reinhold Publishing Company New York (1943) E Pacsu, Advances in Carbohydrate Chem, 1, 78 (1945).

[28] E Fischer, M. Bergmann and A. Rabe, Ber., 53, 2362 (1920)

Dale.[29] An explanation for this behavior was arrived at simultaneously by Freudenberg and E. Braun and by Bott, Haworth and Hirst.[30] The evidence adduced by the latter workers for the structure of the mannose derivative (I) follows:

Substance with analysis corresponding to that for methyl tetraacetylmannoside (I) $\xrightarrow{OH^-}$ Substance with analysis corresponding to that for methyl monoacetylmannoside (II)

$\xrightarrow{(CH_3)_2SO_4}{NaOH}$ Trimethyl derivative of (II) $\xrightarrow{\% HCl}$ Trimethyl mannose $\xrightarrow{Br_2}$ 3,4,6-Trimethyl mannonic delta lactone

The structure of the lactone was demonstrated by synthesis. Eliminating a three-atom ethylene-oxide ring as improbable, the position of the alkali-resistant acetyl group is located by this evidence as carbon 2. The resistance of this group to alkaline hydrolysis is indicative of an unusual structure An orthoester structure agrees with this resistance as well as with the ease with which the acetyl and methyl groups are hydrolyzed by acids. The ordinary alkyl orthoacetates exhibit similar properties.

Methyl orthoacetate

Compound I
3,4,6 Triacetyl-D-mannose
methyl 1,2-orthoacetate

These compounds were called originally "gamma" glycosides (a term used for furanosides at the time) because of their ease of hydrolysis by acids, but it seems desirable to follow the suggestion of Haworth, Hirst and Stacey[31] and to name them as derivatives of orthoacetic acid. Sugars for which orthoacetate derivatives have been reported are: rhamnose, mannose, lyxose, 4-D-mannose β-D-glucoside, talose, ribose, α-glucoheptose, turanose, maltose, fructose, neolactose and α-guloheptose.[32]

[29] J. K. Dale, J. Am. Chem Soc , 46, 1046 (1921)
[30] K Freudenberg and E. Braun, Naturwissenschaften, 18, 393 (1930), H G Bott, W N. Haworth and E. L Hirst, J Chem. Soc , 1395 (1930)
[31] W N. Haworth, E L. Hirst and M Stacey, J Chem Soc , 2864 (1931)

The mechanism and necessary conditions for orthoester formation have been investigated by Frush and Isbell.[32] The principal necessary condition seems to be that the halogen atom of the acetylglycosyl halide from which the orthoester is obtained and the acetyl group on the adjacent carbon should lie on opposite sides of the pyranose ring. Details of this mechanism and of the preparation of these compounds are described in a later paragraph.

An interesting reaction useful in testing for the orthoester structure is that which takes place with hydrogen chloride in chloroform. Under these conditions, the orthoester derivatives are converted to the normal 1-halogeno acetyl sugars.[32]

Sugar methyl orthoacetate   Acetylglycosyl chloride
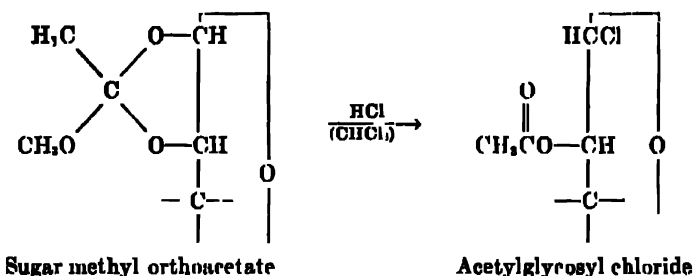
Derivatives having an orthoacetic acid structure (with a free hydroxyl rather than a methoxyl group) have been described,[33] but no direct proof was provided. The orthoacetic acid and ester structures call for a new asymmetric carbon, but the expected isomers have never been obtained.

A monobenzoyltalose is the only known benzoate ester having a probable orthoester or orthoacid structure.[34] The following structure has been provisionally assigned to this substance:
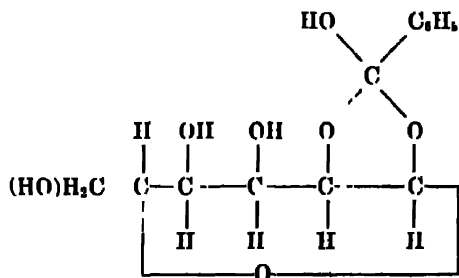
In contrast to the properties of orthoacetic *esters*, the monobenzoyltalose

---

[32] For detailed references see: H. L. Frush and H. S. Isbell, *J. Research Natl. Bur. Standards*, **27**, 413 (1941); also, E. Pacsu.[27]

[33] W. N. Haworth, E. L. Hirst and E. G. Teece, *J. Chem. Soc* , 1408 (1930); N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc* , **58**, 2534 (1936).

[34] W. W. Pigman and H. S. Isbell, *J. Research Natl. Bur. Standards*, **19**, 189 (1937).

is relatively stable to acids and is unstable in the presence of traces of alkali.

E. Acetyl Migration. In the presence of dilute alkali, acyl groups attached to sugars which also contain free hydroxyls may wander and occupy new positions. Helferich and Klein[35,35] observed a mutarotation to take place for solutions of 1,2,3,4-tetraacetyl-β-D-glucose and found that the soft-glass container catalyzed the transfer of an acetyl group probably from the fourth to the sixth carbon atom. When the resulting 1,2,3,6-tetraacetylglucose was partially methylated by methyl iodide and silver oxide, a second migration of an acetyl group from carbon 1 to carbon 4 took place. Such migrations[36] have been observed frequently and are considered to take place through an intermediate orthoacid, as suggested by Fischer,[37] rather than by an actual hydrolysis and recombination of the wandering group. It should be noted that the geometry of the pyranose rings is such that groups attached to carbons 1,4 and 6 can approach each other quite closely (see p. 54, Fig. 1) and that the postulated six-membered orthoacetic structures are strainless even when the two linkages are *trans* to the ring.
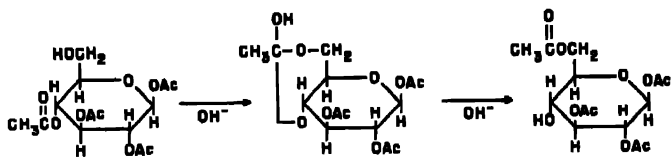
1,2,3,4-Tetraacetyl-   Intermediate orthoacetic   1,2,3,6-Tetraacetyl
glucose   acid   glucose

2. Acetylglycosyl Halides (Halogeno Acetyl Sugars)

A. Cyclic Forms. The acetoxy group on the reducing carbon of the acetylated sugars (carbon 1 of the aldoses and carbon 2 of ketoses) can be replaced by halogen atoms. The resulting compounds, the acetyl glycosyl halides (also called the halogeno acetyl sugars and acetohalogeno sugars), are very important intermediates for the synthesis of sugars and their derivatives.

$X = F, Cl, Br, I$
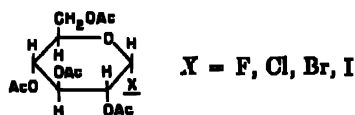
Tetraacetylglucosyl halide

[35] B. F. Helferich and W. Klein, *Ann.*, *450*, 219 (1926); *455*, 173 (1927).
[36] See review by E. L. Hirst and S. Peat, *Ann. Reports Chem. Soc.*, *31*, 172 (1934)
[37] E. Fischer, *Ber.*, *53*, 1624 (1920).

The first compound of this type (tetraacetylglucosyl chloride) was prepared by Colley by the action of acetyl chloride on glucose.[38] It may also be considered that the acetylglycosyl halides are derivatives of anhydro polyhydric alcohols, so that tetraacetylglucosyl bromide is also 1-C-bromo-tetraacetylpolygalitol, and, in fact, polygalitol (1,5-anhydrosorbitol) has been obtained therefrom by indirect replacement of bromine by hydrogen (see under Polygalitol). The high reactivity of the halogen is typical of that displayed by α-halogeno ethers. Like the latter, the acetylglycosyl halides are discussed under derivatives of aldehydes.

The most important methods for the preparation of these substances follow.

a. ACTION OF A CONCENTRATED SOLUTION OF HYDROGEN HALIDE IN ACETIC ANHYDRIDE OR IN GLACIAL ACETIC ACID ON THE ACETYLATED SUGAR;[39] $X = Cl, Br, I$

The glacial acetic acid solution is most commonly employed, but the acetic anhydride solution offers some advantages. The latter solution, actually a solution of acetyl bromide in glacial acetic acid, has a lower vapor pressure and freezing point than HBr in glacial acetic acid; also a higher concentration of the HBr (40-42 per cent) may be obtained.

b. ACTION OF LIQUID HYDROGEN HALIDE ON THE ACETYLATED SUGAR[40]

This method is especially valuable for obtaining the acetylglycosyl fluorides, a reaction investigated particularly by Brauns.[41] In some instances (cellobiose, see page 431), the hydrogen fluoride causes rearrangements to take place with the production of derivatives of new sugars. Prolonged action of hydrogen bromide produces dibromides by replacement of the acetoxy groups at carbons 1 and 6 (anomeric and primary-alcohol groups).

c. ACTION OF PCl₅ AND AlCl₃

A mixture of phosphorus pentachloride and aluminum chloride is frequently used for preparing the acetylglycosyl chlorides from the acetylated sugars in chloroform solution.[42] Titanium tetrachloride may be used

[38] M. A. Colley, *Ann chim phys.*, [4] *21*, 363 (1870); D. H. Brauns, *J. Am. Chem. Soc.*, *44*, 401 (1922)

[39] A. Bodart, *Monatsh.*, *23.* 1 (1902); E. Fischer and H. Fischer, *Ber.*, *43*, 2530 (1910).

[40] E. Fischer and E. F. Armstrong, *Ber.*, *34*, 2885 (1901).

[41] D. H. Brauns, *J. Am. Chem. Soc.*, *45*, 833 (1923); D. H. Brauns and H. L. Frush, *J. Research Natl Bur. Standards*, *6*, 449 (1931).

[42] F. v. Arlt, *Monatsh*, *22*, 144 (1901); Z. H. Skraup and R. Kremann, *ibid.*, *22*, 375 (1901).

in place of the mixture of aluminum and phosphorus chlorides.[42] However, some sugars (cellobiose and lactose) undergo partial racemization of the ring carbons during the reaction with the phosphorus and aluminum chlorides and yield the halides of new sugars (see page 431).

Colley's method, using acetyl chloride, is not widely employed because of the difficulty in controlling the reaction. The first method is the best for most preparations although for the fluorides the second method is particularly valuable. The stability of the acetylglycosyl halides follows the order: fluorides > chlorides > bromides > iodides. The iodides decompose rapidly even at 0°C. whereas the fluorides may be kept for long periods without decomposition.

As the carbon atom carrying the halogen atom is asymmetric, two isomers are possible. Application of the Isorotation Rules of Hudson indicates that most of these compounds belong to a single series which is assigned the alpha configuration.[44] Schlubach, however, has reported that α-tetraacetylglucosyl bromide (dextrorotatory) can be converted into the anomeric β-tetraacetylglucosyl chloride (levorotatory) by treatment with silver chloride. The beta isomers are very unstable and are rapidly transformed into the ordinary alpha isomers[45] (dextrorotatory). The instability of the beta isomers makes it difficult to use them for the synthesis of glycosides and other compounds by the Koenigs-Knorr reaction (see below). But Hickinbottom[46] has found that the β-glucosyl halides with an unsubstituted hydroxyl on carbon 2 (3,4,6-triacetylglucosyl chloride) or with a trichloroacetyl group on carbon 2 (2-trichloroacetyl-3,4,6-triacetylglucosyl chloride) are fairly stable.

Alkalies remove halogen and acetyl groups from the acetylglycosyl iodides, bromides and chlorides. However, the fluorine atom in tetraacetyl glucosyl fluoride is more stable than the acetyl groups which may be removed by alkali leaving glucosyl fluoride.[47] The fluorine is more easily removed by acids than by bases, a relation which is the reverse of that for other halogens and most acyl groups. By heating gentiobiosyl fluoride with water and calcium carbonate, the free sugar is regenerated.

[42] E. Pacsu, Ber., 61, 1508 (1928)
[44] When the rotations of the acetylglycosyl bromides are compared with those of the fully acetylated sugars, it is found that for the D series nearly all the bromides are more dextrorotatory than the corresponding acetates. Although this tells nothing of the absolute configuration, it does divide the halides into series having the same configuration.
[45] E. Fischer, Ber., 44, 1898 (1911); H. Schlubach, ibid., 59, 840 (1926); P. Brigl and H. Keppler, ibid., 59, 1588 (1926); D. H. Brauns, J. Am. Chem. Soc., 49, 3170 (1927)
[46] W. J. Hickinbottom, J. Chem. Soc., 1676 (1929).
[47] B. Helferich, K. Bäuerlein and F. Wiegand, Ann., 447, 27 (1926).

The importance of the acetylglucosyl halides lies in the ease with which the halogen atom may be replaced by many acoxyl, aroxyl, and alkoxyl groups. Ordinarily the reaction is carried out in an anhydrous inert solvent (e.g., benzene) with alcohols or the silver salts of phenols or acids and in the presence of silver carbonate, silver oxide or an organic base such as pyridine or quinoline. These latter substances probably function by removing the halide ion as AgX or the hydrogen halide as the salt of the organic base. When water is formed in the reaction, the presence of a desiccant in the reaction mixture is desirable. Powdered "Drierite" (anhydrous calcium sulfate) is particularly good for this purpose.[48] The reactivity of the acetylglycosyl halides is in the order: I > Br > Cl > F. Since the iodides decompose too easily to be kept for any time and since the fluorides react with too much difficulty, the bromides and chlorides are most commonly employed.

These reactions of the acetylglycosyl halides may be formulated as follows.

| 1-Acyl-2,3,4,6 tetra-acetylglucose | Tetraacetylglucosyl bromide | Alkyl tetraacetyl-β-glucoside |
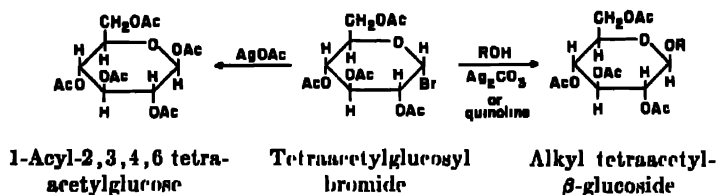
Some of the many acyl groups (see the above formulas) which have been introduced into the sugar molecule by this means are: CH_3CO—,—NO_2, Cl_3CCO—, —PO_4H_2, —SO_3H, $CH_3C_6H_4SO_2$—(tosyl), C_6H_5CO—, etc. A few of the alkyl and aryl groups (R in the above formulas) are: CH_3—,C_2H_5—, $C_{16}H_{33}$—, CH_2OHCH_2—, C_6H_5— , benzyl, α-naphthyl, menthyl, glucosyl, etc.[49] When water is present (e.g., aqueous acetone), the bromine is replaced by a hydroxyl group and the product, tetraacetylglucose in the above example, mutarotates since the reducing carbon has a free hydroxyl group.

The mechanism of the halogen-replacement reaction has been discussed by Frush and Isbell.[50] In general, the product usually obtained by replacement of the halide atom by another group has a beta configuration for the anomeric carbon (carbon 1 of the aldoses). Since the acetylglycosyl hal-

[48] D. D. Reynolds and W. L. Evans, J. Am. Chem Soc., 60, 2559 (1938), introduced the use of the substance for this purpose after Helferich had used the less-efficient calcium chloride. B. Helferich and J. Goerdeler, Ber., 73, 532 (1940), have studied the optimal proportions of reagents and the effect of different dehydrating agents on the yield of several glycosides produced by this reaction.

[49] Additional details of the application of the method to the preparation of the glycosides may be found on p. 193.

[50] H. L. Frush and H. S. Isbell, J. Research Natl. Bur. Standards, 27, 413 (1941).

ide from which it is made most probably has the alpha configuration, a change of the configuration of carbon 1 has taken place, a process generally known as a Walden inversion. This inversion is most simply explained by assuming that, as the halide ion with the pair of electrons forming the halide-carbon bond dissociates, the entering alkoxyl group with a pair of electrons forms a new bond on the opposite side of the carbon from the side at which the halogen departs.

$$
\begin{array}{ccc}
\text{H} & \text{H} & \text{H II} \\
\text{CH}_2\text{O:} \quad \text{C:Br} & \text{CH}_2\text{O: C:Br} & \text{H}^+ \ \text{H} \\
 & & \text{CH}_2\text{O:C} \qquad :\text{Br}^- \\
\text{HCOAc} & \text{HCOAc} & \text{HCOAc} \\
-\text{C}- \quad \text{O} & -\text{C}- \quad \text{O} & -\text{C}- \quad \text{O}
\end{array}
$$

| Tetraacetylglucosyl bromide (*alpha*) | Reaction intermediate (activated state) | Methyl tetraacetyl-glucoside (*beta*) |
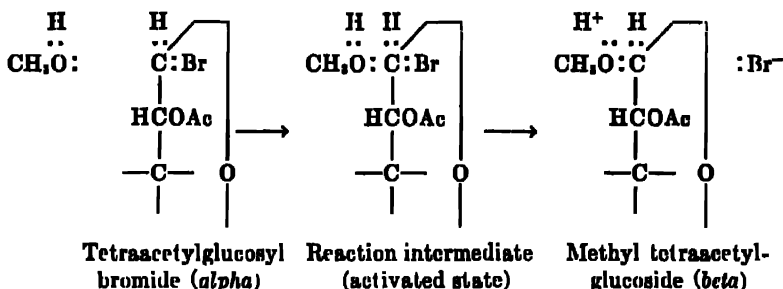
When the halogen and the acetyl group on the second carbon are on opposite sides of the pyranose ring a competing reaction is possible and methyl orthoacetates of the sugars also are formed.[50]

$$
\begin{array}{ccc}
\text{II} & \text{H} & \text{H} \\
\text{CH}_2\text{O:} & \text{O C:Br} & \text{II} \quad \text{H} \\
 & \text{CH}_3\text{COCH} & \text{CH}_2\text{O} \ \ \text{O:C:Br} \\
 & -\text{C}- \quad \text{O} & \text{CH}_3 \quad \text{O CH} \\
 & & -\text{C}- \quad \text{O}
\end{array}
$$

| Tetraacetylmannosyl bromide | Reaction intermediate | Triacetylmannose methyl 1,2-orthoacetate |
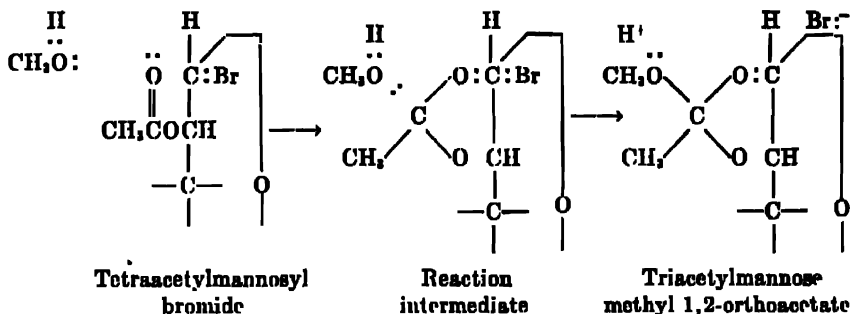
Although the structures of the acetylglycosyl halides are not rigorously established, all the evidence indicates that in their preparation from the fully acetylated sugars, particularly by the IIX-glacial acetic acid method, the ring structures of the acetates are maintained. Since the halides are in turn convertible to the acetyl glycosides and by deacetylation to the free glycosides, all by reactions in which a ring change has never been observed, the ring structures of all these compounds are determined by that of the related glycoside of known structure. Most of the known acetylglycosyl halides appear to be pyranoses, but from pentaacetylgalactofuranose a tetraacetylgalactosyl bromide has been prepared which reacts to give an ethyl galactofuranoside.[51]

⁴¹ H. H. Schlubach and K. Meisenheimer, *Ber.*, **67**, 420 (1034).

B. Optical Rotation and Atomic Dimension. D. H. Brauns[52] has made a very careful and extensive investigation of the optical rotatory relationships of the acetyl glycosyl halides and of the active alkyl halides.[53] An interesting relation was found to exist between the differences in the specific rotation of acetylglycosyl halides of the same sugar and the corresponding differences in the covalent atomic distance between the halogens and the carbon atom. The differences in atomic radii: (Cl, C) − (F, C): (Br, C) − (Cl, C): (I, C) − (Cl, C) are assigned the average values 41:·10:21, respectively The differences between the specific rotations of the tetraacetylglucosyl fluoride, chloride, bromide and iodide in the same order as previously given are 76.0:31 7·39 6 For purposes of comparison, the reduced difference is calculated by making the Cl-F rotational difference 41 and then calculating the other differences from the observed ratios For the glucose derivatives, the reduced rotational differences are· 41 0·17.1 21 1 The corresponding arabinose, xylose and fructose deriva tives show similar close agreement with the atomic dimension differences, but the mannose derivatives give the abnormal ratio, 41.0:24 9:35.2, for the reduced rotational differences. This deviation is ascribed to interaction of the acetyl group on carbon 2 with the hydrogen atom on carbon 1 in the mannose series This interaction may be prevented in the glucose series by attraction of the acetyl group by the ring oxygen

Disaccharides with alpha linkages exhibit similar relations to those for the acetyl glycosyl halides, but disaccharides with beta linkages show some deviations unless the Cl F differences are excluded

C. Acyclic Analogs of the Acetylglycosyl Halides.

Aldehydo acetyl sugars (I) add acetyl halides to form 1-halogeno derivatives (II). These compounds in many ways react analogously to the cyclic acetylglycosyl halides. Application of the Koenigs-Knorr reaction (reaction with alcohols in presence of silver carbonate) leads to the production of hemiacetals (III) and loss of acetyl halide.[54]

aldehydo-Pentaacetyl   1-Bromo-*aldehydo*-   *aldehydo*-D-Galactose ethyl
galactose   D-galactose hexaacetate   hemiacetal pentaacetate

(I)   (II)   (III)
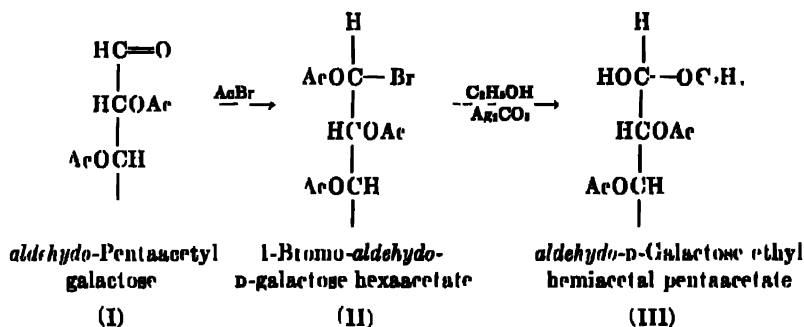
[52] D. H. Brauns, *J. Research Natl. Bur. Standards*, 7, 573 (1931); *J. Am. Chem. Soc.*, 51, 1820 (1929).

[53] D. H. Brauns, *J Research Natl. Bur. Standards*, 18, 315 (1937); *ibid.*, 31, 83 (1943).

[54] M L. Wolfrom, M Konigsberg and F. Moody, *J Am. Chem. Soc.*, 62, 2343 (1940)

A similar series of straight-chain 1-halides are made by treating the fully acetylated hemiacetals with aluminum chloride in chloroform solution.[54, 55]

$$\text{RO-}\overset{\overset{\displaystyle H}{|}}{\underset{|}{C}}\text{-X}$$

The structure of carbon 1 may be represented by RO-C-X. The halogen atom, X, is more reactive than that in the straight-chain 1-acetyl compounds (II), but the reactions of the two types of derivatives seem to be similar.

3. Benzoyl Derivatives

The Schotten-Baumann reaction (action of benzoyl chloride and sodium hydroxide) has been used for benzoylating the hydroxyl groups of carbohydrates.[55] Since the product obtained in this manner is usually a mixture of partially benzoylated sugars, the method ordinarily is modified by the use of benzoyl chloride and pyridine or quinoline.[57] Benzoylation takes place with more difficulty than acetylation, and considerably longer reaction periods are required.

D-Glucose   Pentabenzoyl D glucoses (alpha and beta)

(Bz = C$_6$H$_5$CO-)
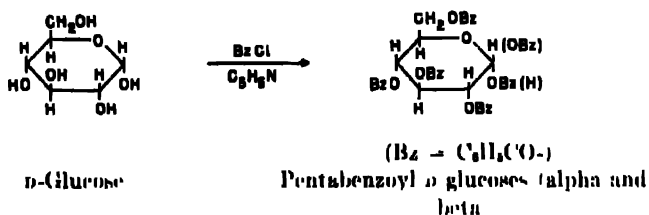
By the use of substituted benzoyl chlorides, derivatives such as the penta-(p-bromobenzoyl)- and penta-(p-nitrobenzoyl)-D-glucoses have been prepared Some of these are colored compounds (p-phenylazobenzoyl esters) and have found use in the chromatographic adsorption method for the separation of constituents of sugar mixtures and for micromanipulations (see p. 133).

The benzoyl sugars are quite similar in their properties and reactions to the acetyl sugars, and it frequently happens that, when a desired acetate ester cannot be obtained in a crystalline condition, the benzoate ester may crystallize. They may be converted to benzoylglycosyl halides by methods similar to those for the acetylglycosyl halides, and the halogen may be replaced by R- O groups (R = alkyl, aryl and acyl groups) as for the

[54] E. M. Montgomery, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **59** 1124 (1937)

[55] Z. H. Skraup, *Monatsh* 10, 305 (1889), L. Kueny, *Z. physiol. Chem.* 14, 330 (1890)

[57] E. Fischer and H. Noth, *Ber.*, 51, 321 (1918); P. A. Levene and G M Meyer, *J. Biol. Chem.*, 76, 513 (1928).

acetyl analogs. As the compounds have not received the same amount of attention that has been devoted to the acetate esters, many problems remain uninvestigated. With the exception of the monobenzoyltalose, which probably has an orthobenzoic acid structure,[58] orthobenzoates are not known.

Wandering of aryl groups from an esterified to an unesterified hydroxyl occurs[59] for benzoyl as well as acetyl groups. For example, D,L-1,4-dibenzoylgalactitol melts at 171 °C., but if held at this temperature the product solidifies to an isomer melting at 202° and which is 1,6-dibenzoylgalactitol.[60] But, in general, benzoyl groups are more stable than acetyl groups. The benzoyl derivatives of the free-aldehyde form of the sugars have been extensively investigated by Brigl and associates[61] and are similar to their acetyl analogs.

The use of boric acid has been suggested for the preparation of partially benzoylated sugars.[62] Unimolar benzoylation of glycosides and mercaptals results usually in preferential esterification of the primary hydroxyl.[63]

Several partially benzoylated sugars and glucosides are naturally occurring. Griebel isolated a monobenzoylglucose (vaccinin) from the juice of blueberries (*Vaccinium Vitis-idaea* L.). It was shown by Ohle[64] probably to be 6-monobenzoyl-D-glucose. Populin, which is found in the bark of a species of poplar, was demonstrated by Richtmyer and Yeakel[63] to be salicyl 6-benzoyl-β-glucoside:
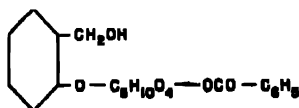
Populin (6-Benzoylsalicin)

A non-reducing dibenzoyldisaccharide containing glucose and xylose residues has been reported[65] as occurring in *Daviesia latifolia*, an Australian shrub.

From the biological standpoint, 1-monobenzoylglucuronic acid is the most important benzoyl derivative. This compound occurs in the urine of

[58] W. W Pigman and H. S. Isbell, *J. Research Natl. Bur. Standards*, *19*, 189 (1937).

[59] H. Ohle, *Ber.*, *57*, 403 (1924); P. Brigl and H. Grüner, *Ann*, *495*, 67 (1932).

[60] R. M Hann, W D. Maclay and C S Hudson, *J Am. Chem. Soc.*, *61*, 2432 (1939).

[61] P. Brigl and H Mühlschlegel, *Ber*, *63*, 1551 (1930).

[62] P. Brigl and H. Grüner[59]; P. Brigl and H. Gruner, *Ber.*, *67*, 1969 (1934).

[63] T. Lieser and R. Schweizer, *Ann.*, *519*, 271 (1935); N. K. Richtmyer and E. Yeakel, *J. Am. Chem. Soc*, *56*, 2495 (1934).

[64] H. Ohle, *Biochem. Z.*, *131*, 611 (1922)

[65] F. B Power and A H. Salway, *J. Chem. Soc.*, *105*, 767, 1062 (1914).

dogs fed benzoic acid. Its structure was shown by the following evidence.[66] Upon acetylation and esterification, the natural product gives a triacetyl methyl ester. This product is identical with that obtained by the reaction of 1-bromo-tetraacetylglucuronic acid methyl ester with silver benzoate and must have the benzoyl group at carbon 1.

4. Galloyl Derivatives and Tannins

Certain tannins are probably gallic acid and digallic acid esters of glucose and of other sugars and derivatives. In order to provide evidence for the structure of the gallotannins, Fischer, Freudenberg and Bergmann[67] synthesized a number of these derivatives by the action of triacetylgalloyl chloride or pentaacetyl-m-digalloyl chloride on glucose. The acetyl groups were subsequently removed. These substances may be represented by the general formula:

Pentagalloyl or pentadi-galloylglucose (galloyl-tannins)

Gallic Acid radical

m-Digallic acid radical
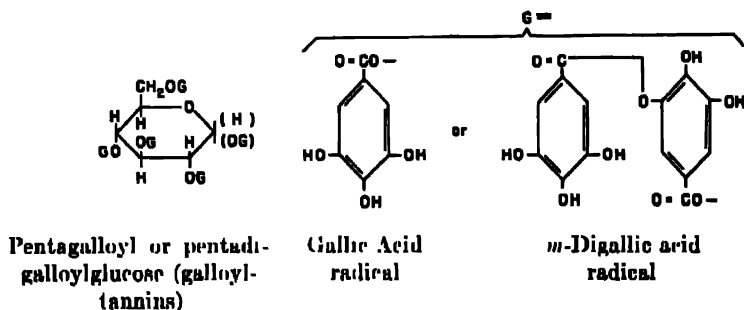
The synthetic galloyl and digalloyl esters were not demonstrated absolutely as identical with the natural gallotannins, but the natural substances may be mixed esters of galloyl and digalloylglucose, in which case the number of isomers would be so great as to make the synthesis extremely difficult. In addition, tri-, tetra- and poly-galloyl radicals might also be present in the molecule. Karrer, Salomon and Peyer[68] suggest that the Chinese gallotannin from the leaf galls of *Rhus semialata* is a mixture. Certain fractions have the average composition of a nonagalloylglucose with the nine galloyl groups attached together or to the sugar residue, possibly as four digallic acid and one gallic acid although other combinations may occur. For the Turkish gallotannin, obtained from gall nuts of certain oaks, the problem appears simpler since the molecule contains only five molecules of gallic acid; the tannin is, presumably, the penta-

[66] W. F. Goebel, *J. Biol. Chem.*, *122*, 649 (1937–38).

[67] E. Fischer, *Ber.*, *52*, 809 (1919); K. Freudenberg, "Tannin, Cellulose and Lignin;" J. Springer, Berlin (1933).

[68] P. Karrer, H R. Salomon and J Peyer, *Helv. Chim. Acta*, *6*, 3 (1923).

galloylglucose. However, the substances are difficult to purify, and the natural substances are clearly mixtures. These structures are questioned by Nierenstein.[69] The tanning properties of synthetic galloyl esters of the sugars are very similar to those of natural gallotannins.[70]

Glucogallin obtained from the Chinese rhubarb by Gilson has been identified[71] through synthesis as 1-galloyl-β-D-glucose.

The bark of the North American shrub *Hamamelis virginica* contains in addition to other tannins, about 1 to 2 per cent of crystalline hamameli-tannin (I).[67] The substance has the composition of a digalloylhexose, and on acid or enzyme hydrolysis, it gives two moles of gallic acid and one mole of an unusual hexose, hamamelose (II). As shown by its reduction to 2-methylpentanoic acid (III), the sugar must have a branched chain[72]

$$\text{(I)} \qquad\qquad \text{(II)} \qquad\qquad \text{(III)}$$
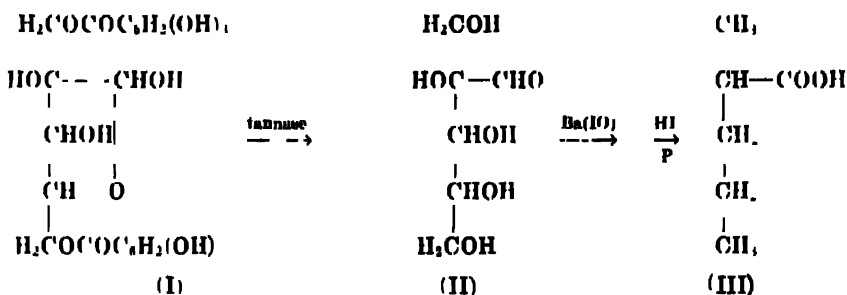
The configuration of carbon atoms 3 and 4 must be the same as in D-ribose and D-arabinose, for hamamelonic acid has been synthesized from D-arabulose by the following procedure:

$$\text{D-Arabulose} \qquad\qquad \text{(IV)} \qquad\qquad \text{(V)}$$
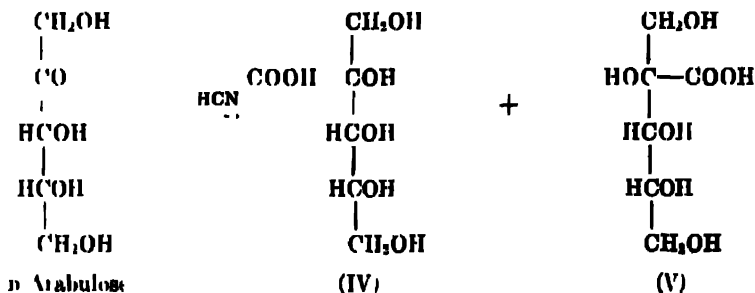
Of the two products formed, one is identical with hamamelonic acid, obtained by the oxidation of the sugar. On the basis of a comparison of opti-

[69] M Nierenstein, C W Spiers and A. C Hadley, *J Am Chem. Soc*, **47**, 1728 (1925)

[70] A. Russell, W G Tebbens and W. F. Arey, *J. Am. Chem. Soc.*, **65**, 1472 (1943), A Russell and W. G Tebbens, *ibid*, **66**, 1866 (1944).

[71] E Fischer and M. Bergmann, *Ber.*, **51**, 1766 (1918).

[72] O Th. Schmidt. *Ann.*, **476**, 250 (1929)

cal rotations, it seems likely that the structure of the acid is given by (V), and hamamelose is 2-C-hydroxymethyl-D-ribose.[73] The only other known branched-chain sugar of natural origin is apiose from the glycoside apiin (see Chapter XI).

5. Other Organic Esters

The sugars and their derivatives have been esterified with many other organic acids.[74] Among these are the fatty acids and cinnamic acid.[75] Most of the products have been made by the action of acyl halides and pyridine on sugars. The fatty acid esters are similar in properties to the natural fats, the glycerol esters.

Considerable interest has been shown in the partially esterified esters of the sugar alcohols and their anhydrides because of their surface active properties. For this reason, the methods of preparation have received much study.[76] (For additional discussion, see under Carbohydrate Inner Ethers.)

The monopalmitate of ascorbic acid[76a] has been prepared by a method[77] that has been applied only to a few other carbohydrates. The method consists of reacting ascorbic acid and fatty acid in 95% sulfuric acid at room temperature. Since the esterification is reported to take place for primary alcohol groups,[76a, 77] the method may have value in the preparation of other pure monoesters. The monoesters of ascorbic acid offer considerable promise as antioxidants for edible fats and oils.[78]

The ease of crystallization and the stability under mild conditions of hydrolysis has created interest in the sugar carbanilates. These derivatives are made by the reaction of carbohydrates with phenylisocyanate in pyridine solution.[79]

$$
\begin{array}{ccc}
| & & O \\
| & C_6H_5N{:}CO & \parallel \\
HCOH & \xrightarrow{\quad\quad} & HCOC{\cdot}NH{\cdot}C_6H_5 \\
| & & |
\end{array}
$$
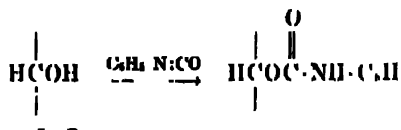
[73] O. Th. Schmidt and K. Heintz, *Ann.*, *515*, 77 (1934)

[74] References to these compounds will be found in the handbooks on sugar chemistry.

[75] K. Hess and E. Messmer, *Ber.*, *54*, 499 (1921), S. Odén, *Arkiv Kemi, Mineral Geol.*, *7*, No. 15 (1918); G. Zemplén and E. László, *Ber.*, *48*, 915 (1915)

[76] H. A. Goldsmith, *Chem. Revs.*, *33*, 257 (1943)

[76a] D. Swern, A. J. Stirton, J. Turer and P. A. Wells, *Oil & Soap*, *20*, 224 (1943).

[77] W. R. Bloor, *J. Biol. Chem.*, *7*, 427 (1910); *11*, 141, 421 (1912).

[78] R. W. Riemenschneider and J. Turer, U. S. Patents 2,383,815-16, Aug. 28, 1945.

[79] H. Tessmer, *Ber.*, *18*, 965 (1885). See also; W. M. Hearon, G. D. Hiatt and C. R. Fordyce, *J. Am. Chem. Soc.*, *66*, 995 (1944). M. L. Wolfrom and D. E. Pletcher, *ibid.*, *62*, 1151 (1940).

Polymerizable esters of sugars are made by treatment of sugars with methacrylic anhydride and pyridine.[80]

$$
\begin{array}{ccc}
| & & | \\
HCOH & & HCOCO{-}C(CH_3){=}CH_2 \\
| & + (CH_2{=}CCH_3CO)_2O \longrightarrow & | \\
HCOH & & HCOCO{-}C(CH_3){=}CH_2
\end{array}
$$
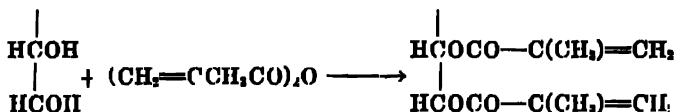
Solutions of glucose pentamethacrylate gel in the presence of cobalt naphthenate or of benzoyl peroxide.

6. Tosyl and Mesyl Derivatives

p-Toluenesulfonic[81] (tosyl), methanesulfonic[82] (mesyl) and other organic sulfonic esters have been prepared. The tosyl esters, which have been particularly well studied, exhibit certain unique characteristics which make them of great importance in synthetic and analytical organic chemistry. Presumably, the other sulfate esters should have analogous properties, but they have not received enough study to make this certain. Preparation of sulfate esters is accomplished by treatment of a carbohydrate with a pyridine solution of an aryl or alkyl sulfonyl chloride (RSO_2Cl) or with 50 percent sodium hydroxide and the sulfonyl chloride at room temperature. Under these conditions, all of the hydroxyl groups may be esterified except those on the reducing (anomeric) carbons which are replaced by halide atoms. Thus, glucose gives tetratosylglucosyl chloride. The primary hydroxyl group seems to be more easily esterified than the secondary hydroxyls.[83]

The tosyloxy groups which esterify primary hydroxyl groups may be replaced by an iodine atom when the ester is heated with an acetone or acetonylacetone solution of sodium iodide. Tosyloxy groups esterified with secondary hydroxyls usually remain unaffected by this treatment unless contiguous to a similar group esterified with a primary hydroxyl.[84] When the latter condition exists, both groups may be removed with the formation of a double bond:

$$
\begin{array}{cccc}
| \; | & | \; | & | & | \\
HC{-}O & HC{-}O & HCOTs & CH \\
| & \xrightarrow{NaI} & | & , & | \quad\xrightarrow[heat]{NaI}\quad || \\
H_2COTs & H_2CI & H_2COTs & CH_2
\end{array}
$$
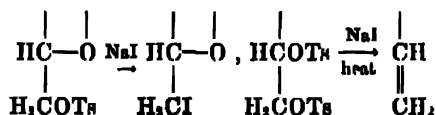
[80] R. H. Treadway and E Yanovsky, *J. Am. Chem. Soc*, **67**, 1038 (1945); W. N. Haworth, H Gregory and L F. Wiggins, *J Chem Soc*, 488 (1946).

[81] K. Freudenberg, O. Burkhart and E. Braun, *Ber.*, **59**, 720 (1926).

[82] B. Helferich and A Gnuchtel, *Ber.*, **71**, 712 (1938); B. Helferich and H. Jochinke, *ibid.*, **73**, 1049 (1940)

[83] A. Bernoulli and H. Stauffer, *Helv. Chim Acta*, **23**, 615 (1940); J. Compton, *J. Am. Chem. Soc*, **60**, 395 (1938).

[84] R. M. Hann, A. T. Ness and C. S. Hudson, *J. Am. Chem. Soc*, **66**, 73 (1944).

Creation of a double bond also may occur when there is a free hydroxyl adjacent to a tosyl group at a primary alcohol grouping as in 6-tosylglucofuranosides.[85] Exceptions to the rule are the tosyl esters of isomannide and isosorbide; the tosyloxy groups of these compounds, although esterifying secondary hydroxyl groups, are replaced with iodine under the above conditions (see under Isomannide).

The difference in case of replacement of tosyloxy groups esterified with primary and secondary alcoholic groups is used to measure quantitatively the primary groups in a compound.[86] This is done by tosylation of the material; treatment of the ester with sodium iodide replaces the tosyl groups esterified with primary alcoholic groups; the iodo compound is treated with silver nitrate, and the iodine atoms are replaced quantitatively with nitrate groups; the liberated iodide precipitates as silver iodide which may be determined quantitatively.

$$
\begin{array}{ccccc}
 & \overset{|}{\underset{|}{O}} & & \overset{|}{\underset{|}{O}} & & \overset{|}{\underset{|}{O}} \\
HC & \xrightarrow[\substack{acetone\\(heat)}]{NaI} & HC & \xrightarrow{AgNO_3} & HC & + AgI \\
CH_2OTs & & CH_2I & & CH_2ONO_2 &
\end{array}
$$
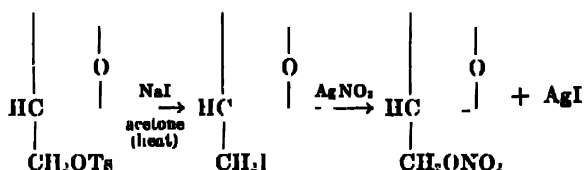
The yield of the iodo compound or of the p-toluenesulfonic acid is high and has been used for the determination of the nature of the alcoholic group in the parent compound.[87] The replacement of a tosyloxy by a nitro group is also brought about directly by heating the ester with silver nitrate in acetonitrile solution. Since the nitro group can be removed with the formation of a free hydroxyl group by reduction with iron dust and glacial acetic acid, mixed acyl derivatives may be prepared (see under Nitrates). The mesyl esters (CH_3SO_2OR) may be carried through a similar series of replacement reactions, and for these esters it is also possible to replace with iodine some of the mesyl groups which esterify secondary hydroxyls.[82]

Secondary tosyl groups are difficult to remove, but the removal may often be accomplished (without the complications mentioned below) by treatment with sodium amalgam in alcoholic solution.[88] By the usual means (alkaline hydrolysis or action of sodium methylate), the deacylation proceeds with difficulty, and Walden inversions take place. Kenyon and Phillips[89] have shown that the alkaline hydrolysis of the tosyl ester of an active alcohol leads to an alcohol with a different sign for the rotation. In contrast the hydrolysis of the acetate yields an alcohol with the same

[85] D. J. Bell, E. Friedmann and S. Williamson, J Chem. Soc., 252 (1937).
[86] J. W. H. Oldham and J. K. Rutherford, J. Am. Chem. Soc, 54, 366 (1932).
[87] W. T. Haskins, R. M. Hann and C S. Hudson, J. Am. Chem Soc., 64, 132 (1942).
[88] K. Hess and K. E. Heumann, Ber., 72, 119 (1939).
[89] J. Kenyon and H. Phillips, Trans. Faraday Soc., 26, 451 (1930).

sign for the optical rotation. These transformations are illustrated for the
active benzylmethylcarbinols.[10] In one of the two hydrolytic reactions an

$(\alpha = +33)$

$$C_6H\ CH_2\ \underset{\substack{|\\CH_3}}{\overset{\substack{H\\|}}{C}} - OH \quad \xrightarrow{CH_3\ C_6H_4\_SO_2Cl}$$

(Ac₂O) ↓

$$C_6H_5CH_2\ \underset{\substack{|\\CH_3}}{\overset{\substack{H\\|}}{C}} - OAc$$

$(\alpha = +71)$

$(\alpha = +31)$

$$C_6H_5CH_2-\underset{\substack{|\\CH_3}}{\overset{\substack{H\\|}}{C}}-OSO_2C_6H_4CH_3$$

KOAc in
alcohol ↓

$$C_6H_5CH_2-\underset{\substack{|\\H}}{\overset{\substack{CH_3\\|}}{C}}-OAc \quad (\alpha = -7.0)$$

OH- ↓

$$C_6H_5CH_2-\underset{\substack{|\\H}}{\overset{\substack{CH_3\\|}}{C}}-OH \quad (\alpha = .32)$$
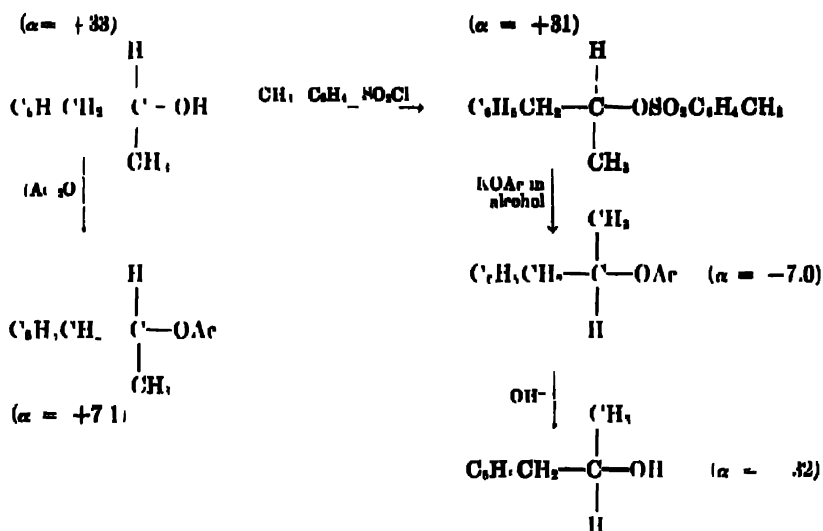
inversion of configuration must have taken place. The change of sign pro-
duced during the hydrolysis of the tosyl ester is probably indicative of a
Walden inversion. The difference between the two reactions lies in the
point at which the hydrolysis takes place. For the acetates, the cleavage

occurs between the acyl group and the oxygen atom $\left(R-O \stackrel{|}{\underset{|}{}} Ac \right)$ while for

the tosyl esters, it takes place between the alkyl radical and the oxygen

bridge $\left(R \stackrel{|}{\underset{|}{}} O \quad Ts \right)$. The Walden inversion takes place when the group

attached immediately to the asymmetric center is removed. Other groups
whose removal are likely to lead to Walden inversions are: halogen, other
sulfate, amino and nitrate.

The tosyl derivatives of the sugars differ from those of the simple alco-
hols in the possible presence of other hydroxyl groups on neighboring
carbon atoms. During the hydrolytic reaction, the oxygen atom of a
neighboring hydroxyl group may compete with that of the hydroxyl groups

[10] H. Phillips, *J. Chem. Soc*, **123**, 44 (1923)

of the solvent in replacing the tosyl group. The intramolecular reaction leads to the production of an anhydro sugar, *i.e.*, a secondary oxygen ring is produced between the carbon to which the tosyloxy group had been attached and a neighboring carbon. Since this ring formation takes place with inversion of configuration, the anhydro sugar is a derivative of a new sugar. Thus, the hydrolysis of the tosyl derivatives of the sugars frequently leads to the formation of derivatives of new sugars. This is of particular importance for the synthesis of the rare sugars and as a possible explanation of certain biological interconversions of the sugars such as the *in vivo* transformation of glucose to galactose and the synthesis of lactose by the mammary gland. The transformation of glucose to galactose has been carried out by the hydrolysis of tosyl derivatives of glucose. Thus, the methyl 2,3-dibenzoyl-4-tosyl-6-trityl-α-D-*gluco*pyranoside yields a 3,4-anhydro*galacto*side which upon hydrolysis is converted to a galactose derivative accompanied by a gulose derivative.[91] Since the anhydro ring may be broken at either oxygen bond and since inversion takes place at the carbon which loses its electron pair, derivatives of two sugars are usually formed in the hydrolysis of the anhydro ring.
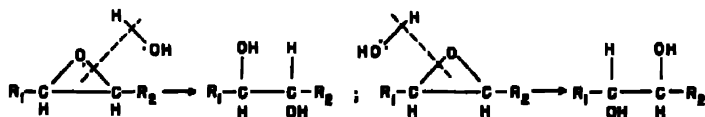
The rare sugar D-altrose may be prepared from D-glucose by the following series of reactions.[92]

Methyl 2,3-ditosyl-4,6-benzylidene-α-glucoside   (I)

↓ NaOEt

Methyl 2,3-anhydro-4,6-benzylidene-α-D-alloside   (II)

↓ KOH

Methyl 4,6-benzylidene-α-D-altroside + Methyl 4,6-benzylidene-α-glucoside

(III)   (IV)

↓ H⁺

Methyl α-altroside   (V)

[91] J. W. H. Oldham and G. J. Robertson, *J. Chem. Soc.*, 685 (1935)

[92] G. J. Robertson and C. F. Griffith, *J. Chem. Soc.*, 1193 (1935); N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, 63, 1727 (1941)

(I)   (II)

KOH

(IV)   +   (III)   ⟶   (V)
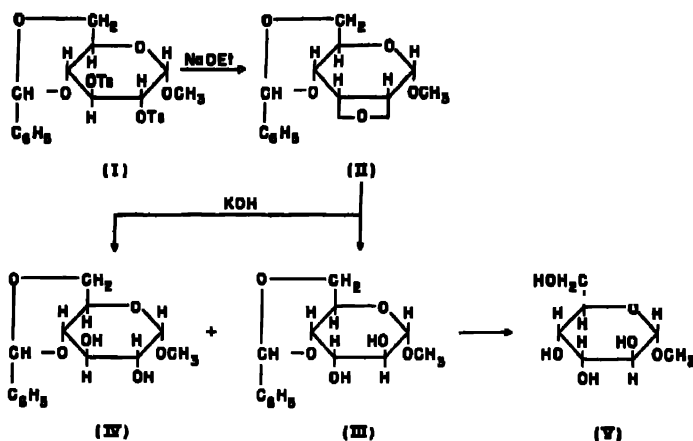
The transformations from one sugar to another which result from the saponification of tosyl esters are of considerable importance for the preparation of the rare sugars. Some of the interconversions which have been carried out are:

D-Glucose to D-gulose and D-galactose derivatives.[91] [93]

D-Glucose to D-altrose derivatives.[92]

D-Glucose to D-idose or L-idose derivatives.[94]

D-Fructose to D-sorbose derivatives.[95]

L-Rhamnose to 6-desoxy-D-allose derivatives.[96]

D-Galactose to D-idose derivatives.[97]

After extensive investigation, Ohle and Schultz[98] concluded that an ethylene-oxide ring is formed during the alkaline hydrolysis of tosyl esters of sugars only when the hydroxyl and tosyl groups on adjacent carbon atoms are *trans* to one another. When this condition is not realized, the compounds are very stable to alkaline hydrolysis.[99] The question of the removal of tosyl groups without simultaneous Walden inversion is not definitely answered, but it seems probable that removal without inversion may take place under severe hydrolytic conditions,[100] particularly when the formation of anhydro rings is impossible. Although the ethylene-oxide

[91] A. Müller, *Ber*, *68*, 1094 (1935).

[94] W. Lake and S. Peat, *J. Chem. Soc.*, 1009 (1939); A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, *29*, 152 (1946).

[95] H. Ohle and F. Just, *Ber.*, *68*, 601 (1935).

[96] P. A. Levene and J. Compton, *J. Biol. Chem.*, *116*, 169 (1936).

[97] L. F. Wiggins, *J. Chem. Soc.*, 522 (1944).

[98] H. Ohle and C. A. Schultz, *Ber.*, *71*, 2302 (1938).

[99] A. Müller, M. Móricz and G. Verner, *Ber.*, *72*, 745 (1939); D. J. Bell and S. Williamson, *J. Chem. Soc.*, 1196 (1938).

[100] G. J. Robertson and D. Gall, *J. Chem. Soc.*, 1600 (1937).

ring is the usual ring formed, other types have been reported. By treating methyl 3-tosyl-triacetyl-β-D-glucopyranoside (I) with sodium methylate, Peat and Wiggins[101] obtained, in addition to methyl 2,3-anhydro- and 3,4-anhydro-β-alloside (II and III), the methyl 3,6-anhydro-β-glucoside (IV)
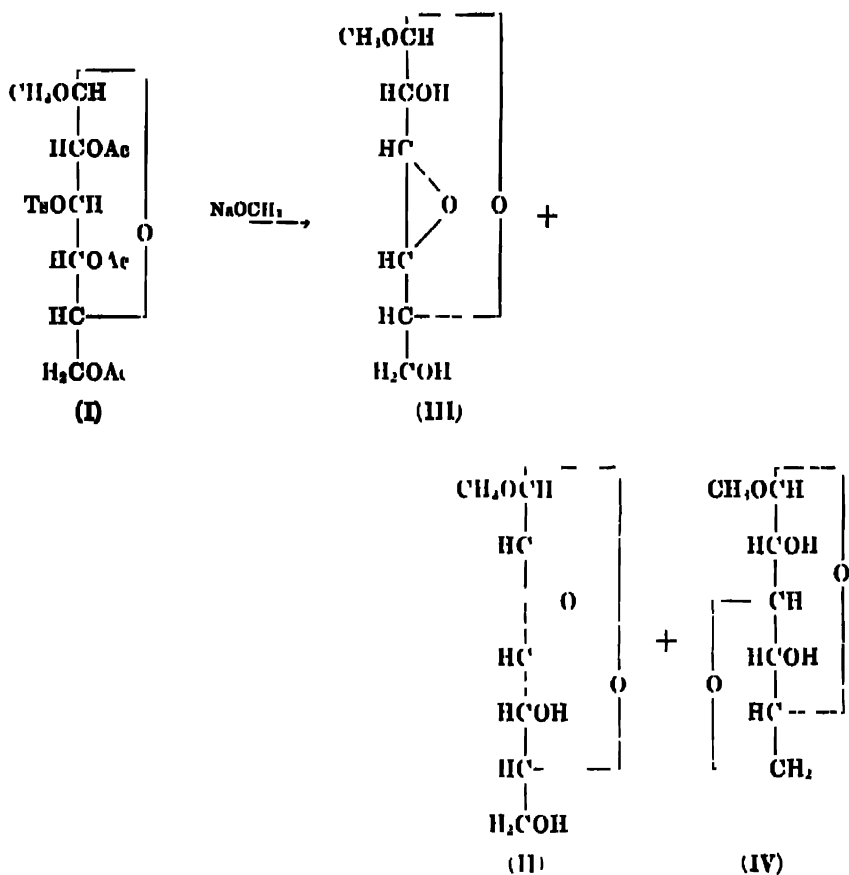
This behavior is in contrast to that of 3-tosyl-monoacetone-glucofuranose which is saponified under the similar conditions without Walden inversion or anhydro ring formation.[102] The difference in the behavior of the two 3-tosyl compounds arises from the presence in the former case of a free hydroxyl adjacent to the tosyl group. The formation of the 3,6-anhydro derivative probably takes place by a further reaction of the pri-

[101] S Peat and L F Wiggins, *J Chem Soc*, 1938, 1810 (1938), W N. Haworth, L N Owen and F Smith, *ibid*, 88 (1941)

[102] H. Ohle and W. Wilcke, *Ber*, 71, 2316 (1938).

mary hydroxyl group at carbon 6 of the anhydroallosides (II or III). Otherwise, it would be necessary to postulate a reaction between the *cis* groups at carbon atoms 3 and 6 of (I) that would not involve a Walden inversion.

INORGANIC ESTERS

1. Nitric Acid Esters

Carbohydrate nitrates have considerable importance as explosives.[103] Cellulose and, to a much lesser extent, starch nitrates are used as smokeless powders. Nitrates of the polyols, particularly glycerol and mannitol, are the most important esters of the lower carbohydrates. In conjunction with nitroglycerin, sucrose octanitrate has found use. (Polysaccharide nitrates are discussed in more detail under the individual polysaccharides.) Because of the difficulty of stabilizing the nitrates of sugars, their use as explosives has not been developed extensively.

The nitrates of simple sugars and glycosides have been prepared by Will and Lenze[104] by allowing a mixture of nitric acid and sulfuric acids to react with sugars or glycosides at 0°C. Nitric acid alone effects only partial nitration. Alpha and beta isomers are prepared in this manner.

$$C_6H_{12}O_6 \xrightarrow[\text{H}_2\text{SO}_4]{\text{HNO}_3} C_6H_7O_6(NO_2)_5$$
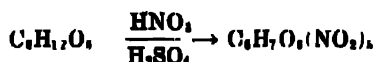
The halogen atoms of acetylglycosyl halides (or of acylglycosyl halides in general) may be replaced with nitrate groups with the production of alpha or beta 1-nitro acetyl sugars.[105] Similarly, halogen atoms or tosyloxy groups esterifying primary alcoholic groups (*e.g.*, 6-halogeno-glucose derivatives) are substituted by nitrate groups by reaction with silver nitrate. The amount of silver halide produced has been used for the estimation of primary alcohol groups (see p. 171) Since the *nitrate* radical may be converted to a hydroxyl by reduction with iron dust and acetic acid (or indirectly for nitrate groups in terminal positions by reaction with sodium iodide) the nitrates are often useful for the preparation of partially substituted sugar derivatves.[88, 106]

Alkaline hydrolysis of nitrate groups takes place preferably in a manner

[103] For a general discussion, see: T. L. Davis, "Chemistry of Powder and Explosives," vol. II, p. 101; John Wiley, New York (1943).

[104] W. Will and F. Lenze. *Ber.*, *31*, 68 (1898); J. A. Wyler, U. S. Patents 2,039,045 and 2,039,046, April 28, 1936

[105] Z. H. Skraup and R. Kremann, *Monatsh.*, *22*, 1043 (1901); W. Koenigs and E Knorr, *Ber.*, *34*, 974 (1901); A. Colley, *Compt. rend.*, *76*, 436 (1873)

[106] D. J. Bell and R. L. Synge, *J. Chem. Soc.*, 1711 (1937); 833, 836 (1938); J. Dewar and G. Fort, *ibid.*, 492, 496, 499 (1944); J. W. H. Oldham, *ibid.*, 2840 (1925).

analogous to that for the tosyl esters; i.e., by replacement of the $-ONO_2$ group rather than the $-NO_2$ groups. Evidence for such a mechanism is given by the formation of methyl 3,6-anhydro-β-glucoside by the alkaline hydrolysis of methyl 2,3,4-triacetyl-6-nitro-β-glucoside. As is also the case for the tosyl derivatives, simple replacement of the nitro group by a hydrogen takes place only when anhydro formation is not possible.[107]

The alkaline saponification of nitrates has been studied particularly for the cellulose nitrates.[108] Marked decomposition occurs, and nitrites, ammonia, cyanides, carbon dioxide, oxalic acid and various hydroxy acids are among the final products. Under reducing conditions, particularly with alcoholic ammonium sulfide, cellulose and other carbohydrate nitrates are smoothly hydrolyzed. It is interesting that one nitrate group of mannitol hexanitrate is removed by the action of dry pyridine.

2. Sugar Carbonates

The sugars react with methyl (or ethyl) chloroformate at 0°C. in the presence of alkali to give mixed carbonate and carbomethoxy (or carboethoxy) esters.[109] The carbonate structures resemble those of the isopropylidene derivatives as shown in the formulas.
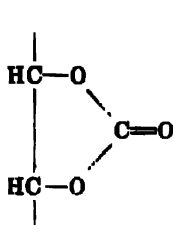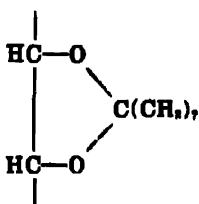
| Carbonate structure | Isopropylidene (acetone) structure | Carboethoxy ester structure |
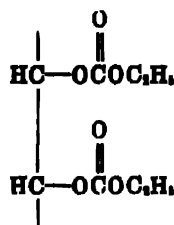
In the presence of pyridine and methyl or ethyl chloroformate, the sugars are converted to fully acylated carbomethoxy or carboethoxy esters.[110]

A better method for the preparation of the carbonates utilizes the action of carbonyl chloride (phosgene) at 0°C. on pyridine solutions of the sugars. By this method, glucose, fructose, mannose and arabinose dicarbonates have been obtained.[111]

The carbonate groups, similarly to other acyl groups, are hydrolyzed easily by alkalies and with more difficulty by acids. This difference from

[107] E. K. Gladding and C. B. Purves, J. Am. Chem. Soc., 66, 76 (1944).
[108] See: J. Barsha, in "Cellulose and Its Derivatives," E. Ott, editor; Interscience Press, New York (1943).
[109] C. F. Allpress and W. N. Haworth, J. Chem. Soc., 125, 1223 (1924)
[110] G. Zemplén and E. D. László, Ber., 48, 921 (1915).
[111] W. N. Haworth and C. R. Porter, J. Chem. Soc., 151 (1930); 2796 (1929).

the properties of sugar acetals (e.g., the isopropylidene sugars) is used advantageously for the preparation of the glucofuranosides (see Chapter V). Because of the hydrolytic action of hydroxyl ions, the methylation procedures cannot be used for the elucidation of the structures of these compounds, but it is usually considered that the sugar carbonates are analogous in structure to isopropylidene sugars (acetone sugars). The dicarbonates of several common sugars have been given the following structures: Glucofuranose 1,2–5,6-dicarbonate, mannofuranose 2,3–5,6-dicarbonate, galacto- and arabino-pyranose 1,2 3,4-dicarbonate, fructopyranose 1,2–4,5-dicarbonate.

A carbonate group and an isopropylidene group may be introduced into a sugar molecule in a single step by carrying out the reaction with carbonyl chloride in dry acetone solution. Xylose under these conditions gives 1,2-isopropylidene-D-xylose 3,5-carbonate although galactose yields diisopropylidene-6-(chloroformyl)-D-galactose.[112] Treatment of the mannose dicarbonate with thionyl chloride gives the 1-chloromannose dicarbonate.

When the sugar molecule contains only one free hydroxyl group, two moles of the sugar derivative may react with one mole of carbonyl chloride.[113] The following formulas illustrate the reaction of 1,2,3,4-tetraacetylglucose and phosgene to form di-(1,2,3,4-tetraacetylglucose) 6-carbonate.
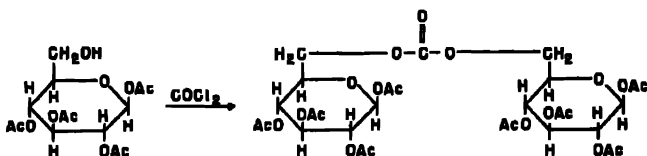
3. Phosphate Esters

The phosphate esters of the sugars are of great biological importance since they act as intermediates in the breakdown of glycogen to lactic acid in the muscle (glycolysis), in the fermentation of sugars to alcohol and other products, and possibly in the oxidative (metabolic) processes in general. They also occur as constituents of nucleic acids and of coenzymes which in turn are closely related to vitamins of the B-complex.

As a group, the phosphoric esters of the sugars are strongly acidic liquids isolated in many instances as the crystalline barium, calcium, lead, sodium or alkaloid salts. They are usually stronger acids than free orthophosphoric acid and neutralize two equivalents of alkali for each phosphate residue. Mixtures of hexose and triose phosphates may be analyzed[114] since the

[112] W N Haworth, C R Porter and A C Waine, *Rec trav chim.*, **57**, 541 (1938).
[113] D Reynolds and W O Kenyon, *J Am Chem Soc*, **64**, 1110 (1942).
[114] K Lohmann, *Ann. Rev Biochem*, **7**, 125 (1938)

latter are hydrolyzed at room temperature by alkali and the former usually by N HCl at 100°C.

Synthesis of Phosphate Esters. The most common method[115] for the *in vitro* synthesis depends on the action of phosphorus oxychloride on sugars at low temperatures and in the presence of a neutralizing agent or catalyst (pyridine, quinoline, carbonates or alkali). To prevent more than one hydroxyl from reacting, it is often necessary to block the other groups. Diacetone-glucose reacts under these conditions to yield a 3-phosphate, which by preferential acid hydrolysis loses the isopropylidene residues to form D-glucose 3-phosphate. The method has been used by Levene and associates[116] in the preparation of many hexose and pentose phosphates. It is reported[117] that the method may be improved by the application of diphenylorthophosphoryl chloride. The resulting reaction takes place in a pyridine solution of hydroxyl-containing substances at room temperature and the diphenyl esters frequently are nicely crystalline substances. The phenyl groups are removed by weak acids or by catalytic hydrogenation employing the Adams-Shriner, platinum-oxide catalyst.

Phosphate esters of hexitol anhydrides have been made by treatment of hexitols with phosphoric acid (see under Carbohydrate Inner Ethers).

Other methods are available for the preparation of the esters substituted at the glycosidic carbon (carbon 1 of aldoses), and these are of particular importance for the synthesis of the important naturally occurring Cori ester. The reaction of silver phosphate with tetraacetylglucosyl bromide introduces the phosphate group at carbon 1; deacetylation gives the glucose 1-phosphate[118] (the so-called Cori ester). This ester also can be prepared by the action of the phosphorylases of potato extracts on starches in the presence of phosphate ions.[119] The enzymic method probably is the best for the purpose.

The crystalline dipotassium salt has been shown by periodic oxidation and synthesis of the beta isomer to be the alpha pyranose isomer.[120] As pointed out by Wolfrom and Pletcher[121] the magnitude of the third disso-

[115] E. Fischer, *Ber.*, **47**, 3193 (1914); C. Neuberg and H. Pollak, *Ber.*, **43**, 2060 (1910).

[116] A. L. Raymond and P. A. Levene, *J. Biol. Chem.*, **83**, 619 (1929)

[117] P. Brigl and H. Muller, *Ber.*, **72**, 2121 (1939).

[118] C. F. Cori, S. P. Colowick and G. T. Cori, *J. Biol. Chem.*, **121**, 465 (1937); F. J. Reithel, *J. Am. Chem. Soc.*, **67**, 1056 (1945).

[119] C. S. Hanes, *Proc. Royal Soc.*, B **128**, 421 (1940); B **129**, 174 (1940); J. B. Sumner and G. F. Somers, *Arch. Biochem.*, **4**, 11 (1944); R. M. McCready and W. Z. Hassid, *J. Am. Chem. Soc.*, **66**, 560 (1944).

[120] M. L. Wolfrom, C. S. Smith, D. E. Pletcher and A. E. Brown, *J. Am. Chem. Soc.* **64**, 23 (1942).

[121] M. L. Wolfrom and D. E. Pletcher, *J. Am. Chem. Soc.*, **63**, 1050 (1941).

ciation constant of orthophosphoric acid is of the same order as that of a polyhydric alcohol, and the glucose 1-phosphate should resemble the glucosides more than the true esters. This resemblance to the glycosides may explain the stability of the Cori ester to alkali. However, the glucose 6-phosphate is less resistant to alkalies The glucose 1-phosphate prepared by Zervas[122] by interaction of tetraacetylglucosyl bromide and silver dibenzylphosphate and subsequent reduction is the beta isomer of the Cori ester.[120] The mannose and galactose 1-phosphates have also been prepared[123] by the Cori procedure

Naturally Occurring Hexose Phosphates. Four hexose phosphates are of particular importance because they are intermediates in the alcoholic fermentation of sugars and in the conversion of glycogen to lactic acid (glycolysis) in muscle tissue. They have been isolated by inhibiting the reactions at the proper stages or by allowing yeast juice to act on sugars in the presence of inorganic phosphates. In the presence of the proper enzymes, they are interconvertible The four are: glucose 1-phosphate (Cori ester—(I)), glucose 6-phosphate (Robison ester- -(II)), fructose 6-phosphate (Neuberg ester—(III)) and fructose 1,6-diphosphate (Harden-Young ester (IV))

HCOPO$_3$H$_2$	HCOH	CH$_2$OH	H$_2$COPO$_3$H
HCOH	HCOH	HC——	HC -
HOCH O	HOCH O	HOCH O	HOCH O
HCOH	HCOH	HCOH	HCOH
HC————	HC———	HC—	HC———
H$_2$COH	H$_2$COPO$_3$H$_2$	H$_2$COPO$_3$H$_2$	H$_2$COPO$_3$H
Cori ester	Robison ester	Neuberg ester	Harden Young ester
(I)	(II)	(III)	(IV)
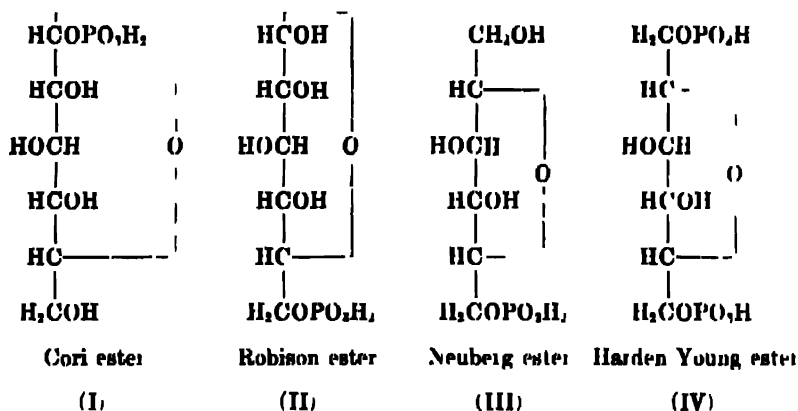
Structure of Harden-Young Ester Fructofuranosides are formed from the fructose diphosphate by treatment with methyl alcohol and hydrogen chloride and subsequent enzymic hydrolysis of the phosphate groups[124] Since the original ester forms a hydrazone readily, and an osazone with the simultaneous loss of a phosphate group, one of the groups is probably

[122] L Zervas, *Naturwissenschaften,* **27**, 317 (1939)

[123] S P Colowick *J Biol Chem ,* **124**, 557 (1938), H Kosterlitz, *Biochem J ,* **33** 1087 (1939)

[124] W. T Morgan and R Robison, *Biochem. J.,* **22**, 1270 (1928)

attached at carbon 1.[125] The other group is probably at carbon 6, for furanosides but not pyranosides may be formed.

Robison Ester and Neuberg Ester.—The Robison ester originally obtained was a mixture of glucose and fructose monophosphates.[126] A glucose 6-phosphate, synthesized by Levene and Raymond,[127] appears to be identical with the aldose portion of the original Robison ester. This conclusion is substantiated by the synthesis of the other isomers: glucose 1-, 3-, 4- and 5-monophosphates.

It seems probable that the ketose portion of the original Robison ester is identical with the Neuberg ester. Since the latter is obtained by partial acid hydrolysis of fructose 1,6-diphosphate, it appears to be fructose 6-phosphate.[128]

Cori Ester The synthesis of this ester, as described above, fixes its structure as α-glucose 1-phosphate

Other Naturally Occurring Esters. Fructose 1-phosphate has been reported to be formed by the glycogenolysis of liver.[128]

The livers of rabbits fed on galactose yield as a result of alkaline hydrolysis a mixture of nonreducing phosphate esters. About 30 per cent is a galactose phosphate. The latter material probably is the galactose 1-phosphate, for its rate of hydrolysis is close to that of the synthetic material.[129]

The action of liver nucleosidase on inosine (hypoxanthine N-riboside) in the presence of inorganic phosphate and xanthine oxidase produces a ribose phosphate which because of its nonreducing properties probably is ribose 1-phosphate.[130] The ester is hydrolyzed easily by acids at room temperature This substance probably plays an important role in the biological synthesis of nucleosides, because it is converted to purine N-ribosides in the presence of purines and enzymes of the phosphorylase type

The phosphate esters of trioses (glyceraldehyde and dihydroxyacetone), glyceric acid and pyruvic acid take part in the transformations of hexoses to lactic acid or ethyl alcohol such as occur in metabolic and fermentation processes [141] Nucleotides, phosphate esters of nucleosides, are products of the partial hydrolysis of nucleic acids and contain phosphorylated D ribose residues (see under Nucleotides)

[125] W Young. *Biochem Z*, 32, 177 (1911); A. v. Lebedev, *ibid*, 36, 248 (1911)
[126] R Robison and E J King, *Biochem J.*, 25, 323 (1931).
[127] P A Levene and A L Raymond, *J Biol. Chem.*, 89, 479 (1930), 91, 751 (1931)
[128] J. Pany, *Z physiol Chem*, 272, 273 (1942)
[129] H W Kosterlitz, *Biochem. J.*, 37, 318 (1943)
[140] H M Kalckar, *Federation Proc.*, 4, 248 (1945)
[141] See J C Sowden and H O L. Fischer, *Ann. Rev. Biochem* 11, 203 (1942), h Lohmann, *ibid*, 7, 125 (1938)

It has been suggested[132] that the occurrence of D-ribose and other rare sugars may be explained by a Walden inversion during the dephosphorylation of another sugar such as is known to take place during the hydrolysis of p-toluenesulfonyl esters of sugars (see Tosyl esters). Although extensively studied, no evidence can be adduced for this view, and studies[133] of the mechanism of the alkaline hydrolysis of phosphate esters indicate that the linkage ruptured is the $\left(P{-}O\right)$ and not the $\left(O{-}R\right)$. The hydrolysis is similar to that of the acetates rather than of the tosyl esters. A more likely source of natural Walden inversions would seem to be the sulfate esters or possibly the disaccharides.

4. Esters of Arsenous Acids

The sodium salt of diisopropylidene-glucose reacts with arsenic tribromide to give a compound that is hydrolyzed to the 3-metaarsenite.[134] Unless the hydrobromic acid is neutralized, one of the acetone groups is removed to give the monoisopropylidene derivative.

5. Sulfate Esters

Esterification[145] results from the reaction of carbohydrates with HSO_4Cl or SO_2Cl_2. Often, chlorine atoms as well as sulfate groups are introduced, but in the presence of pyridine and at low temperatures chlorination can be prevented. The degree of esterification increases with increase in temperature. The sulfate esters are amorphous products, which can be obtained in a solid state as the salts of alkaline earth elements or of alkaloids. Some of the salts have been crystallized.

When fructose is heated under pressure with bisulfite solutions, nonreducing fructose sulfonic acids (C—S linkage) are obtained which may be separated as crystalline brucine salts.[136] Such sulfonic acid derivatives of the sugars are of importance because they occur in the products of wood hydrolysis by the action of acids and bisulfites. In addition, sugar bisulfites are formed which have their maximum stability in the pH interval 4 to 7.

[132] R. Robinson, *Nature*, *120*, 41 (1927).

[143] J. Herbert and E. Blumenthal, *Nature*, *144*, 248 (1939); E. E and E. G. V. Percival, *J. Chem. Soc.*, 875 (1945).

[134] P. J. Daughenbaugh, U S. Patent 2,032,263, Feb. 25, 1936.

[135] P. Claesson, *J. prakt Chem.*, [2] *20*, 17 (1879); C. Neuberg and L. Liebermann, *Biochem. Z.*, *121*, 326 (1921); B. Helferich, A. Löwa, W. Nippe and H. Riedel, *Z. physiol Chem.*, *198*, 141 (1923); *Ber*, *56*, 1083 (1923); E. G V Percival and T. H Soutar, *J. Chem. Soc.*, 1475 (1940).

[146] E Hägglund, H. Heiwinkel and T. Bergek, *J. prakt. Chem.*, [2] *162*, 2 (1943)

Since both types of derivatives are not fermentable by yeasts, they must be given a treatment with alkali before the hydrolysis products may be used for fermentation purposes.

Cartilage, tendons, vitreous humor, corneas and other materials con-containing the so-called mucins, mucoids and glycoproteins have a considerable content of carbohydrates and combined sulfur. Acids which have been isolated from these products have been shown by Levene and associates[127] to be sulfate esters of oligosaccharides which yield glucuronic acid and glucosamine or galactosamine on hydrolysis. Galactose sulfates probably are constituents of brain lipides (see under Galactose). Polysaccharide sulfate esters such as heparin are described in Chapter XV. It is probable that these esters are quite widely distributed since many sources of enzymes which hydrolyze hexose sulfates are known.[138]

The alkaline hydrolysis of the sulfate esters seems to proceed differently than for the corresponding tosyl derivatives. Instead of ethylene oxide rings, 3,6-anhydro rings preferentially are formed on removal of the sulfate group, and no inversion of configuration takes place. Thus, whereas 1,2-monoisopropylidene-glucofuranose 6-p-toluenesulfonate gives 1,2-monoisopropylidene-5,6-anhydroglucose after treatment with sodium methylate, the corresponding 6-sulfate gives the 3,6-anhydro derivative after treatment with alkali. However, the corresponding 3-sulfate is hydrolyzed without the formation of an anhydro ring.[139]

6. Boric Acid Esters [*]

Boric acid esters of the sugars are rather ill-defined but v. Vargha reported that when glucose is shaken in an acetone solution containing sulfuric acid and orthoboric acid (H_3BO_3) a crystalline 1,2-isopropylidene-glucofuranose 3,5-orthoborate separates.[140] In the main, however, these esters are used for special purposes. They were used by Böeseken in his classical work establishing the configuration of carbon 1 of glucose (Chapter II) and have been employed for the preparation of partially benzoylated sugar derivatives. In the presence of aqueous borax, the sugar alcohols and sugars have rotations quite different from those for the aqueous solutions.[141a] The enhancement of the rotations in the presence of borates is of particular importance for the alcohols which otherwise have only small rotations.

[127] P A Levene, "Hexosamines, Their Derivatives, and Mucins and Mucoids"; Monograph No 18, The Rockefeller Institute for Medical Research, New York (1922).

[138] Cl. Fromageot, *Ergeb. Enzymforsch.*, 7, 50 (1938)

[139] E. G V. Percival, *J Chem Soc*, 119 (1945).

[140] L. v. Vargha, *Ber*, 66, 704 (1933).

[141a] L. Vignon, *Compt rend*, 77, 1191 (1873), 78, 148 (1874); M. Murgier and M E. Darmois, *Atti X congr. intern. chim.*, 2, 737 (1938).

[*]For additional discussion, see p. 253.

1,3-Butylene glycol and similar glycols react with boric acid, and the products formed can be distilled. The composition and properties of the products show that borate esters are formed.

$$
\begin{array}{ccc}
\text{CH}_2\text{OH} & & \text{CH}_2\text{O} \\
| & & | \qquad \text{\textbackslash} \\
\text{CH} & \xrightarrow{\text{H}_3\text{BO}_3} & \text{CH}_2 \qquad \text{B—OH} \\
| & & | \qquad \text{/} \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{O}
\end{array}
$$
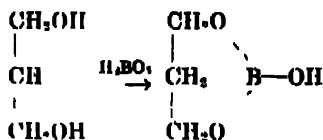
In the case of glycerol, triesters may be formed, and for diethylene glycol linear polymerization seems to occur.[141b]

Boric acid promotes the condensation of α- or β-glucose to products containing as many as ten or more dextrose units.[142] The reaction proceeds in the dry state when the sugar is blended with 5 percent of metaboric acid and heated at 135°C. although the appearance of the crystals does not change. Apparently the boric acid does not enter into the combination, for it can be removed from solutions of the product. The nature of the reaction is not known.

7. Halogeno Esters

Halogeno sugars other than the acylated glycosyl halides (discussed earlier in this chapter) have received only limited study. Halogen esters formed from the primary alcohol group of sugars can be made by several methods, examples of which are given:

1. The prolonged action of liquid HBr on pentaacetylglucose to yield the 1,6-dibromo derivative.[143]

2. The splitting of the anhydro ring of triacetyl-levoglucosan (1,6-anhydroglucopyranose) with PHr$_5$ to give 1,6-dibromo-triacetyl-glucose[144] (see also under Carbohydrate Inner Ethers).

3. The replacement with iodine of p-toluenesulfonyl (tosyl) groups or nitrate groups by reaction with sodium iodide in acetone or acetonyl-acetone solution (see under Nitric Acid and Tosyl Esters).

Halogen esters formed from the primary alcohol can be reduced to give "methyloses" which have a terminal methyl group rather than a primary alcohol group.

Other types of halogen esters are formed by the addition of halogens or halogen acids to unsaturated derivatives such as glycals and by the reaction of sulfurylchloride with glycosides.[145]

[141b] R. E. Rippere and V. K. LaMer, *J. Phys. Chem*, **47**, 204 (1943).

[141] G. J. Leuck U S Patent 2,375,564, May 8, 1945.

[143] E. Fischer and E. F Armstrong, *Ber.*, **35**, 836 (1902).

[144] P. Karrer and A P. Smirnoff, *Helv. Chim. Acta*, **5**, 124 (1922).

[145] E Fischer, M. Bergmann and H. Schotte, *Ber*, **53**, 509 (1920); B. Helferich, G. Sprock and E. Besler, *Ber*. **58**, 886 (1925)

Iodo derivatives of sugars and glycosides (including disaccharides) have shown some promise as therapeutic agents. Following intravenous injection, some of these derivatives are rapidly excreted through the kidney in concentration sufficient to render the kidney and allied structures more opaque to X-rays than surrounding tissue. 6-Iodogalactose and methyl 6-iodoglucoside show particular promise for this purpose.[146]

Halogeno esters of the polyols and anhydrides are discussed elsewhere (p. 252, 281 and 363).

[146] A. L. Raymond and E. Schroeder, U. S. Patent 2,365,777, Dec. 26, 1944; 2,365,776, Dec. 26, 1944.

GLYCOSIDES, FULL ACETALS AND THIOACETALS

The carbonyl group of sugars may react externally with alcohols to form glycosides (mixed acetals) or full acetals of the open-chain form. If the condensation is internal, glycosans are formed which are internal glycosides. Mercaptals and thioglycosides are formed when mercaptans are employed instead of alcohols. These formal relationships are shown in the accompanying formulas:

HC·OR | HCO | HC(OR)₂

$$\text{HC·OR} \quad\quad \text{HCO} \quad\quad \text{HC(OR)}_2$$

$$\overset{\text{ROH}}{\longleftarrow} \quad\quad \overset{\text{ROH}}{\longrightarrow}$$

HC·OR ⌐	HCO	HC(OR)₂
(HCOH)ₙ O ← ROH ·	(HCOH)ₙ ROH →	(HCOH)ₙ
HC ⌐	HCOH	HCOH
CH₂OH	CH₂OH	CH₂OH

Glycoside   Full unmixed acetal

(Full mixed acetal)

H		HC(SR)₂	HCSR
┌─C─┐	−H₂O ←	(HCOH)ₙ	(HCOH)ₙ O
O (HCOH)ₙ O	RSH →	HCOH	HC
HC───	or,	CH₂OH	CH₂OH
CH₂			

Glycosan   Mercaptal   Thioglycoside

(Internal acetal or glycoside)
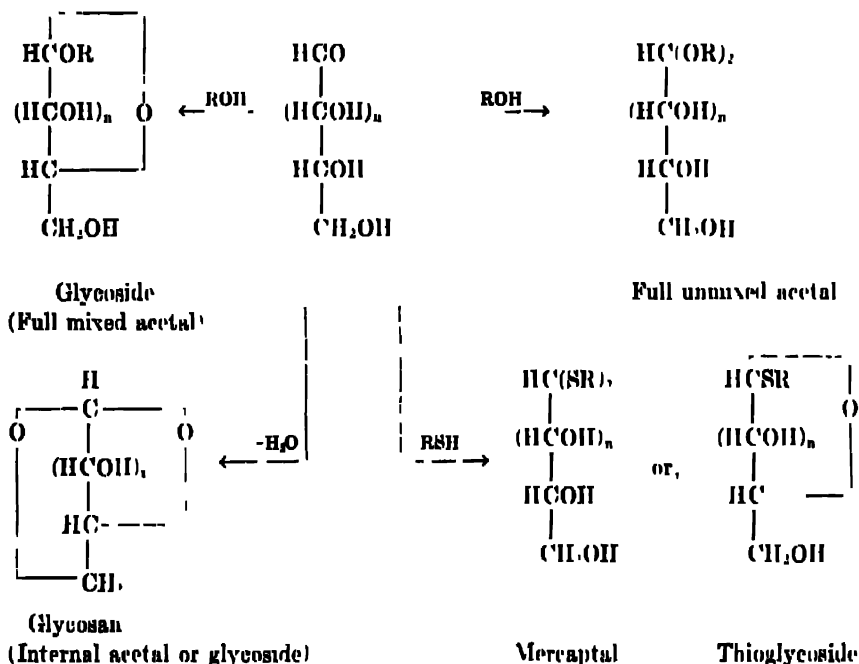
The carbonyl groups of aldehydes or ketones condense with pairs of hydroxyls provided by a carbohydrate to produce alkylidene or arylidene derivatives. These resulting compounds are cyclic acetals or ketals, which

HCOH		HCO┐	
(HCOH)ₙ + RCHO ⟶		(HCOH)ₙ CHR	
HCOH		HCO┘	

(n = 0 or 1)
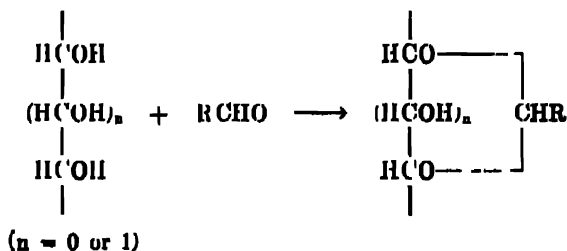
differ from glycosides and glycosans in that the carbonyl group is supplied by a noncarbohydrate.

Anhydro derivatives may be considered to be of two general types: (1) glycosans (inner glycosides) for which water is split out between an anomeric hydroxyl group and a primary or secondary alcohol group, (2) anhydrides (ethers), considered under Ethers in a subsequent chapter, which may be considered to be derived by the removal of water between primary and/or secondary hydroxyl groups.

$$
\begin{array}{ccccc}
\text{HCOH} & & \text{HCOH} & & \text{CH} \\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} \\
\text{CH} \quad O & \longleftarrow & \text{HOCH} \quad O & \rightarrow & \text{HOCH} \quad O \\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} \\
O \quad \text{HC} & & \text{HC} & & \text{HC} \\
\text{CH}_2 & & \text{CH}_2\text{OH} & & \text{CH}_2
\end{array}
$$

Glucose 3 6-anhydride   D-Glucose   β Glucosan
(3,6 anhydroglucopyranose)   (1,6 anhydroglucopyranose)
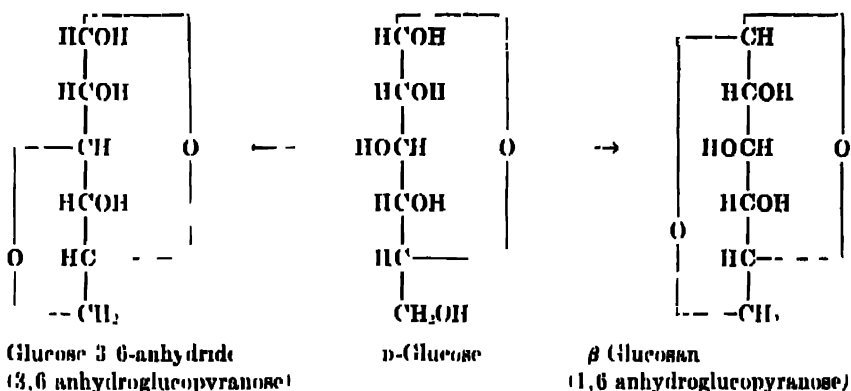
The most important of these derivatives are the glycosides, for they are widely distributed in plants and to a lesser extent in animals. Because the chemistry of the natural glycosides resides to a considerable degree in the noncarbohydrate portion and in biochemical aspects, the natural glycosides are discussed in a later chapter in connection with enzymes (Chapter XI).

1. Glycosides

The glycosides may be defined as acetal derivatives of the cyclic forms of the sugars (normally pyranoses and furanoses) in which the hydrogens of the hemiacetal hydroxyls have been replaced by alkyl or aryl groups and which on complete hydrolysis yield a mono- or poly-hydric alcohol or phenol and one or more monosaccharides. In the older literature the term "glucoside" was used generically for all such derivatives and was not confined to glucose derivatives. Present usage restricts "glucosides" to the glucose derivatives whereas "glycosides" is used in the generic sense. Specific glycosides are named by replacing the ending "ose" of the parent sugar by "oside" and by prefixing the name by the alkyl or aryl radical and the symbol α or β to designate the configuration of the glycosidic (anomeric) carbon, e.g., methyl β-glucopyranoside. For more complex groups, it is sometimes more convenient to use the name of the alcohol or phenol rather than the radical, e.g., hydroquinone α-galactoside. Many phytochemical

names such as salicin and helicin are in use for natural glycosides although chemical names usually are to be preferred since they indicate the structure and facilitate classification. However, the shorter names offer the advantage of brevity and frequently indicate the source of the glycoside, e.g., salicin from *Salix*. For convenience, the alkyl or aryl group is often referred to as the "aglycon group" (less preferably as the "aglucone group") and the corresponding free phenol or alcohol as the "aglycon" (or "aglucone"). The sugar radical is the "glycosyl" group.[1]

Di-, oligo- and poly-saccharides have glycosidic linkages and are to be included among the class of "glycosides." For these compounds, the aglycon group is a sugar radical (see under Oligosaccharides, p. 427). Glycosans are inner glycosides for which acetalization has taken place completely within a single sugar molecule.

A. Methods For Synthesis. The first successful synthesis of glycosides was carried out by the American chemist Arthur Michael. The synthesis was accomplished by the interaction of tetraacetylglucosyl chloride and the potassium salts of phenols.[2] Under the conditions of the reaction, acetyl groups are also removed, and the glucoside is produced.

(Methylarbutin)
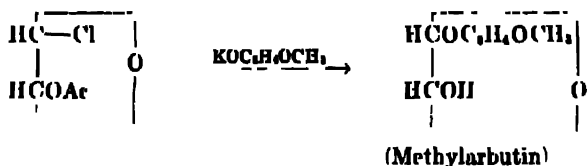
Fischer, in an attempt to synthesize the acetals of the sugars by the action of methyl alcohol and hydrogen chloride, found that only one methyl group was introduced per mole of sugar and that he had obtained the methyl analog of the natural glycosides.[3] The Michael synthesis can be applied only to condensations with phenols and the Fischer synthesis applies only to alcohols. Koenigs and Knorr, however, by utilizing their tetraacetylglucosyl bromide, silver carbonate and an alcohol or phenol (under anhydrous conditions) provided a procedure applicable to the preparation of both alkyl and aryl glycosides.[4] The above methods, their modifications and new methods have been widely applied to the preparation of glycosides. These methods are considered separately below in more detail.

[1] The term "glycosyl" will be used for the sugar radical obtained by removing the hydroxyl of the anomeric carbon (carbon 1 of aldoses). "Glycosido" will be used to refer to the radical obtained by removing the hydrogen from the hydroxyl group of the anomeric carbon.

[2] A. Michael, *Am. Chem. J.*, *1*, 307 (1879); *6*, 336 (1885); *Compt. rend.*, *89*, 355 (1879).

[3] E. Fischer, *Ber.*, *26*, 2400 (1893); *28*, 1145 (1895).

[4] W. Koenigs and E. Knorr, *Ber.*, *84*, 957 (1901).

a. FISCHER METHOD

Aldehydes and ketones react in anhydrous alcoholic solutions of hydrogen chloride with the formation of acetals and ketals, and the simplest members of the sugar series, glycolaldehyde and glyceraldehyde, react similarly. The cyclic sugars, which are already hemiacetals, under these conditions establish an equilibrium in which the alpha and beta pyranosides and furanosides predominate, and probably the true acetals, glycosans and oligosaccharides are present in small amounts. γ-Hydroxy aldehydes such as γ-hydroxyvaleraldehyde, act similarly and create oxygen rings by intramolecular acetal formation.[⁵] The furanose forms of the sugars seem to react the most readily, but the pyranosides are the principal constituents under equilibrium conditions. Hence, if the furanosides are particularly desired, the reaction is carried out under mild conditions at room temperature. At the boiling temperature of the solvent, equilibrium is usually attained after 3 to 24 hours for hydrogen chloride concentrations of 0.5 to 1 5%
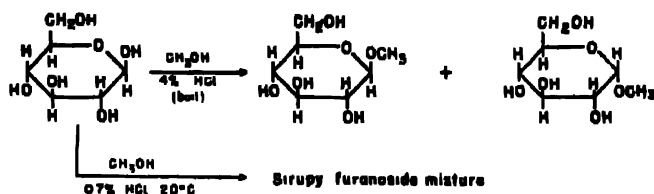
Levene, Raymond and Dillon[⁶] have made a detailed study of the changes which take place during methyl glycoside formation, and their data are summarized for a number of common sugars in Figs 1, 2 and 3

The composition of the reaction mixture was determined by analysis for reducing sugars before and after hydrolysis under strongly acidic conditions (pyranosides and furanosides hydrolyzed) and under weakly acidic conditions (furanosides hydrolyzed).

As will be noted from Fig. 1, furanosides appear to be formed in the first stages of the reaction, but their quantity decreases in the latter stages. On the other hand, the proportion of pyranosides increases progressively with time. The quantity of furanoside varies greatly with the nature of the sugar and seems to be particularly great for ribose. The values for fructose, and possibly other sugars, should be interpreted with caution because the difference in ease of hydrolysis of some furanosides and pyranosides is small (see later discussion of ease of hydrolysis of glycosides).

It seems probable that the dialkyl acetals are formed under the same

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[⁵] B Helferich and F A Fries, *Ber* , **58**, 1246 (1925)

[⁶] P A Levene, A L Raymond and R T Dillon, *J Biol Chem* , **95**, 699 (1932)

conditions as for the glycosides but that the equilibrium favors the formation of the mixed acetals (the glycosides). In methyl alcohol which contains hydrogen chloride, the glucose and galactose dimethyl acetals yield the corresponding pyranosides.[7] For the glucosides, mannosides and galactosides, the alpha pyranose form predominates over the beta in the equilibrium mixture.
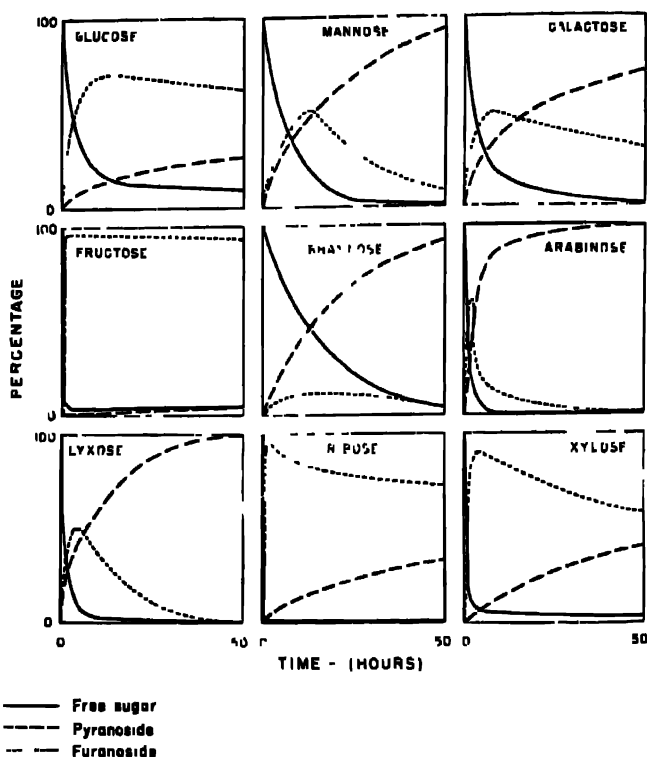
(After Levene, Raymond and Dillon)

FIG. 1. Composition of solution during glycoside formation at 25°C. in methyl alcohol containing 0 5 per cent hydrogen chloride

The Fischer procedure is particularly good for the preparation of the alkyl pyranosides although a few crystalline furanosides have been obtained in this manner.[8] Better methods for the furanosides are described later. The disaccharides are partially hydrolyzed under the conditions of glycoside formation as are also the acetyl groups of acetylated sugars. Since the acetylated sugars are more soluble in alcohols than the free

[7] H. A. Campbell and K. P. Link, *J. Biol. Chem*, *122*, 635 (1938); M. L Wolfrom and S. W Waisbrot, *J. Am. Chem. Soc.*, *61*, 1408 (1939).

[8] E. M Montgomery and C S Hudson, *J Am. Chem. Soc*, *59*, 992 (1937).

sugars, they may be the best materials to use for the preparation of the glycosides of difficultly soluble sugars.

Acid catalysts other than hydrogen chloride that have been suggested[*]
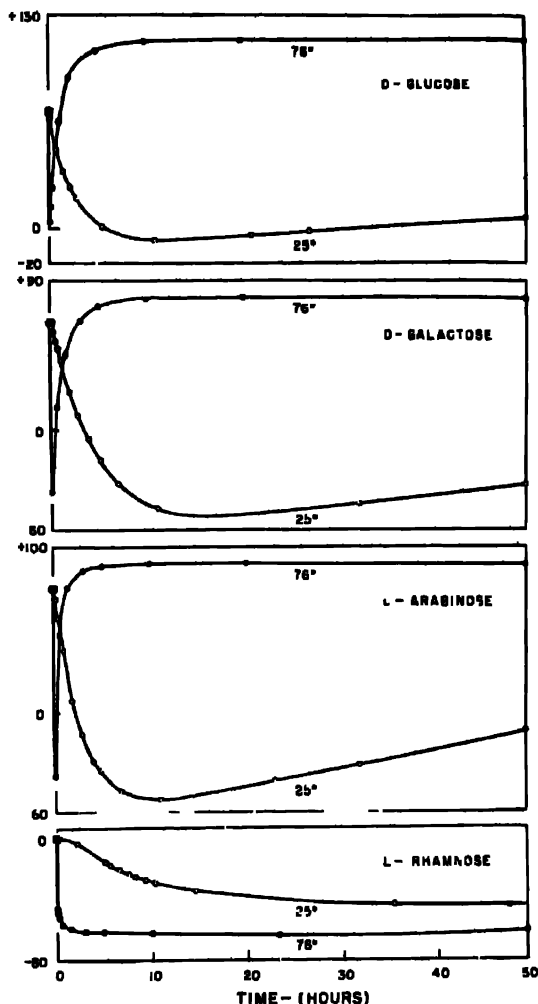
(After Levene, Raymond and Dillon)

Fig 2   Changes in the rotatory power ([α]ᴅ) of solution of sugars in methyl alcohol containing 0 5 per cent of hydrogen chloride (t = 25° or 76°C).

for glycosidification are: sulfuric acid alone or with added bisulfate or phosphoric acid, phosphoric acid alone or with added acid phosphate, perchloric acid, organic sulfonic acids such as benzenesulfonic acid and

[*] See. A Chwala, U. S. Patent 2,356,505, Aug 22, 1944

naphthalenesulfonic acid, and some carboxylic acids such as oxalic and
trichloroacetic acid. Still others are: dimethyl sulfate, iodine, dodecyldi-
methylsulfonium iodide, ammonium chloride, ammonium thiocyanate,
piperidine hydrochloride, hexadecylpyridinium bromide, boron trifluoride,
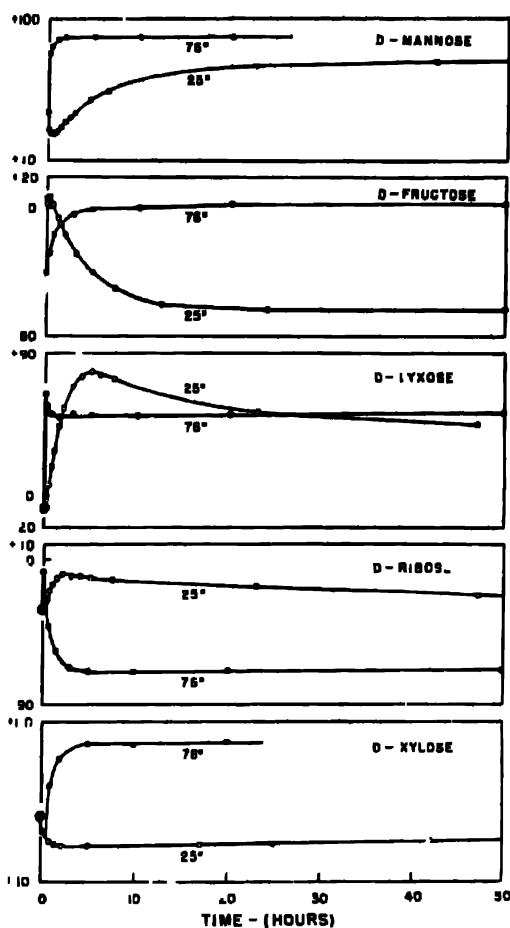
(After Levene, Raymond and Dillon)

FIG. 3 Changes in the rotatory power ($[\alpha]_D$) of solutions of sugars in methyl alcohol
containing 0 5 per cent of hydrogen chloride ($t = 25°$ or $76°C$)

sulfanilic acid and sulfamic acid [10] These catalysts are used in concentra-
tions from 0.001 to 1.0% by weight of the sugar employed.

Because of the difficulty of reaction between long chain alcohols and
sugars, it may be preferable to make glycosides containing active halogen

[10] P L Salzberg and J H Werntz, U S Patent 2,374,236, April 24 1945

atoms (*e.g.*, 2-chloroethyl glucoside) and replace the chlorine atom by reaction with long-chain aliphatic acids or with phenols or amines.[9]

$$Gl\!-\!OCH_2\!-\!CH_2Cl + R\!-\!COONa \rightarrow Gl\!-\!OCH_2\!-\!CH_2OCOR$$

b. MICHAEL SYNTHESIS

As mentioned above, Michael in his original synthesis of the aromatic glycosides utilized the reaction between tetraacetylglucosyl chloride and the potassium salts of phenols. The utility of the method has been greatly increased by using the more reactive tetraacetylglucosyl bromide and by carrying out the reaction in an alkaline aqueous-acetone solution of the phenol.[11] This method is probably the most convenient one for the preparation of phenyl glycosides, and yields the beta isomers. (For exceptions see under Arabinose and under Acetylglycosyl halides). Under the conditions of the modified procedure, the acetyl groups are not saponified, and the acetylated glycosides are obtained.

c. KOENIGS-KNORR REACTION

This method is particularly suitable for the preparation of β-glycosides. It involves treatment of an acetylglycosyl halide with the corresponding alcohol or phenol, in certain inert solvents when necessary, and in the presence of excess silver carbonate or silver oxide.

Pentaacetylglucosyl bromide   Methyl tetraacetyl-β-glucoside
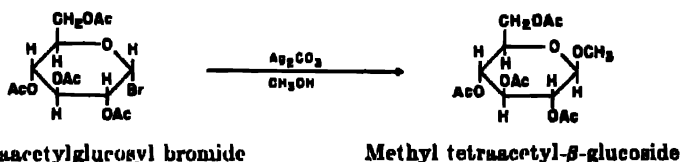
When the halogen atom on carbon 1 and the acetyl group on carbon 2 have a *trans* configuration, the alkyl orthoacetate is usually the main product but some of the alpha and beta isomers are also produced. The mechanism of the reaction is discussed at another place. (See under Acetylglycosyl halides).

Many improvements in the original method, in special instances, are particularly valuable.[12] The use of "Drierite" (anhydrous calcium sulphate) is often beneficial. The presence of iodine may improve the yields. The acetylglycosyl bromides react at a lower temperature than the corre-

[11] C. Mannich, *Ann.*, *394*, 223 (1912); E. Fischer and E. F. Armstrong, *Ber.*, *34*, 2885 (1901); *35*, 833 (1902); J. H. Fisher, W. L. Hawkins and H. Hibbert, *J. Am. Chem. Soc.*, *62*, 1412 (1940).

[12] D. D. Reynolds and W. L. Evans, *J. Am. Chem. Soc.*, *60*, 2559 (1938); B. Helferich and J. Goerdeler, *Ber.*, *73*, 532 (1940); C McCloskey, R. Pyle and G. H. Coleman, *J. Am. Chem. Soc.*, *66*, 349 (1944).

sponding chlorides and are to be preferred for most reactions, e.g., the longer-chain aliphatic alcohols do not react with the chlorides under the usual conditions. If the aglycons are valuable substances, the use of an excess of the glycosyl halide and of both silver oxide and quinoline is advisable.[13] The benzoylglycosyl bromides often may be used advantageously in place of the acetyl analogs.[14]

The previous methods based on the Koenigs-Knorr synthesis give the beta isomer almost exclusively in most instances when the halogen of carbon 1 and the acetyl of carbon 2 have a *cis* relationship, but variations may be introduced so that appreciable quantities of the alpha isomer are produced.[15] The application of mercuric acetate or of sublimed ferric chloride in place of silver carbonate enables one to control the amount of the alpha isomer by fixing the ratio of the halide to the catalyst. It has also been demonstrated that the utilization of quinoline at 100°C. instead of silver carbonate favors the formation of the phenyl α-glycosides.[16]

Since the reaction takes place with Walden inversion, it would seem applicable to the preparation of α-glycosides if carried out with the acetylated β-glycosyl halides rather than with the common alpha isomers. This procedure has succeeded in several instances,[17] but the instability of the ordinary acetylated β-glycosyl halides limits its application. When the β-glycoside is not obtained by this procedure, it is probable that interconversion of the isomeric halides takes place more rapidly than the replacement reaction (see Acetylglycosyl halides).

d. HELFERICH METHOD

The acetoxy group on the first carbon of the acetylated aldoses is more labile than the other acetoxy groups and, as previously discussed (p. 162), is easily replaced by a halogen atom to form the acetylglycosyl halides. It is replaceable, as discovered by Helferich and Schmitz-Hillebrecht,[18] by a phenoxy group when the acetylated sugar is heated with a phenol in the presence of an acid catalyst (zinc chloride or p-toluenesulfonic acid).

$$
\begin{array}{ccccc}
\text{C}_6\text{H}_5\text{OCH} \\
\text{|} \\
\text{HCOAc}
\end{array}
\text{O}
\quad \xleftarrow[\text{POCl}_3]{\text{C}_6\text{H}_5\text{OH,}} \quad
\begin{array}{c}
\text{HCOAc} \\
\text{|} \\
\text{HCOAc}
\end{array}
\text{O}
\quad \xrightarrow[\text{ZnCl}_2]{\text{C}_6\text{H}_5\text{OH}} \quad
\begin{array}{c}
\text{HCOC}_6\text{H}_5 \\
\text{|} \\
\text{HCOAc}
\end{array}
\text{O}
$$
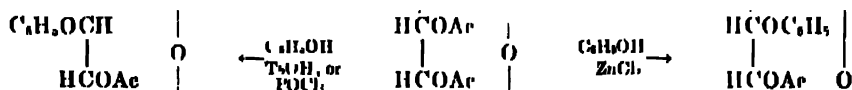
[13] See: A. Robertson and R. B. Waters, *J. Chem. Soc.*, 2730 (1930).

[14] J. W. H. Oldham, *J. Am. Chem. Soc*, 56, 1360 (1934).

[15] G. Zemplén and Z. Csürös, *Ber.*, 64, 993 (1931); G. Zemplén, *Fortschritte Chem. organ. Naturstoffe*, 1. 1 (1938); *Ber.*, 74 A, 75 (1941).

[16] E. Fischer and L. v. Mechel, *Ber.*, 49, 2813 (1916).

[17] W. J Hickinbottom, *J. Chem. Soc.*, 1676 (1929); W. F. Goebel, F. H Babers and O. T. Avery, *J. Exptl. Med.*, 55, 761 (1932).

[18] B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, 66, 378 (1933).

The method is particularly valuable for the preparation of the phenyl
α-glycosides, but unless the optimal conditions are found, considerable
quantities of both isomers are produced.[19] However, the use of p-toluene-
sulfonic acid and a short heating time seems to favor the formation of the
beta isomer whereas zinc chloride and a longer heating time favors the
production of the alpha isomer. The yields may be improved by removing
the acetic acid by vacuum distillation during the reaction.[20a] The phenyl
α-glucoside tetraacetate is the main product when equilibrium is estab-
lished in the presence of zinc chloride and phenol. It is also possible to
convert the acetylated methyl glycosides to the phenyl glycosides under
these conditions.[20b] (A modification of the method employs moist phos-
phorus oxychloride as a catalyst.[21]) Synthesis of the glucosides of long chain
monohydric alcohols by this method has been claimed by H. Bertsch and
G. Rauchalles.[22]

e. USE OF SUGAR MERCAPTALS

Through variation of the conditions employed for the removal of the
thioalkyl groups from sugar mercaptals (by the action of mercuric salts),
pyranosides, furanosides, thioglycosides or acetals may be obtained.[23]
The method is particularly suitable for obtaining furanosides which are
formed under mild conditions especially in the absence of acids (presence
of HgO). In aqueous solutions, the removal of a single thioalkyl group
produces thioglycosides. In most instances, the acetals are formed when
the hydroxyl groups are acetylated or otherwise blocked. The accompany-
ing diagram illustrates the conditions employed for obtaining some of the
possible products from galactose diethyl mercaptal.

HC(SC₂H₅)₂		HC(OCH₃)₂
HCOAc		HCOAc
AcOCH	HgCl₂ CH₃OH , CaCO₃ 70–80°C	AcOCH
AcOCH		AcOCH
HCOAc		HCOAc
H₂COAc		H₂COAc
Pentaacetylgalactose diethyl mercaptal		Pentaacetylgalactose dimethyl acetal
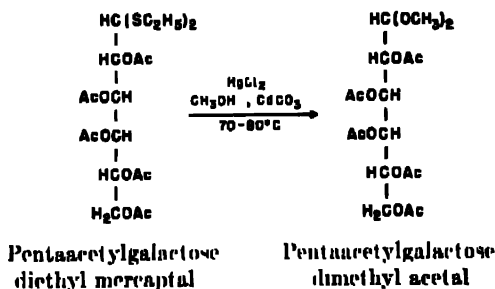
[19] R. T. Williams, *J. Chem. Soc.,* 1402 (1940).

[20] a. K. Nisido, *J. Soc. Chem. Ind. Japan,* 39, 217B (1936); b. E. Montgomery, N.
K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.,* 64, 690 (1942).

[21] T. H. Bembry and G. Powell, *J. Am. Chem. Soc.,* 64, 2419 (1942).

[22] H. Bertsch and G. Rauchalles, U. S. Patent 2,049,758, Aug. 4, 1936

[23] W. Schneider, J. Sepp and O. Stiehler, *Ber.,* 51, 220 (1918), J. W. Green and E.
Pacsu, *J. Am. Chem. Soc.,* 59, 1205, 2569 (1937); 60, 2288 (1938); M. L. Wolfrom, L.
Tanghe, R. George and S. Waisbrot, *ibid.,* 60, 132 (1938).

Ethyl α-galactopyranoside   Galactose diethyl   Ethyl β-galactofuranoside
                                        mercaptal

Ethyl α-galactofuranoside   Ethyl α-thiogalactofuran
                                               oside
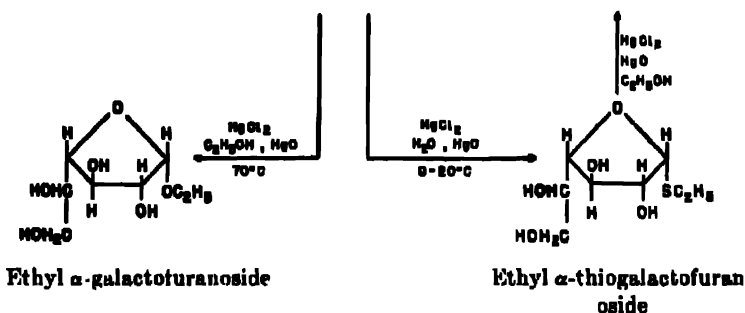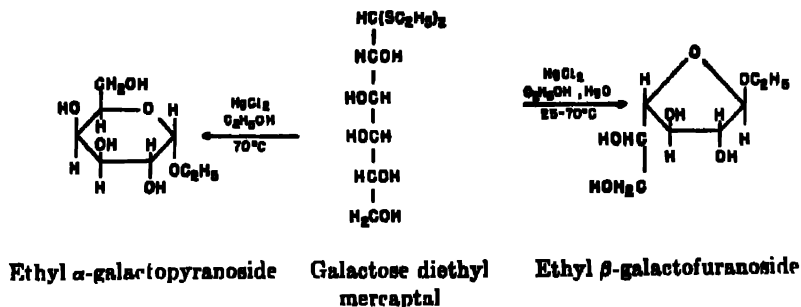
The α-thiogalactofuranoside was not isolated, but the corresponding glucose derivative was made by the procedure shown.

f. DIRECT ALKYLATION METHOD

The glycosidic hydroxyl undergoes preferential alkylation when the sugar is alkylated with one equivalent of dimethyl sulfate and alkali.[34] The procedure is particularly valuable for obtaining methyl glycosides of the mannose type for which the Koenigs-Knorr reaction fails because of orthoester formation. Alkylation of tetraacetylfructose with silver oxide and methyl iodide leads to the methyl fructoside tetraacetate.[35]

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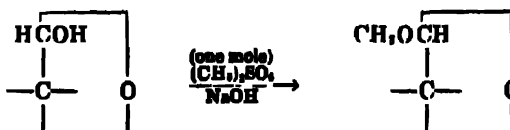
[34] L. Maquenne, *Bull. soc. chim.*, [3] *33*, 469 (1905); H. S. Isbell and H. L. Frush, *J. Research Natl. Bur. Standards*, *24*, 144 (1940).

[35] C. S. Hudson and D. H. Brauns, *J. Am. Chem. Soc.*, *38*, 1216 (1916).

g. From Glycals

Alcohols in the presence of perbenzoic acid add to the double bond of glycals to give glycosides of two sugars (see under Glycals) since two new asymmetric carbons are produced (1 and 2). For the addition of methyl alcohol to glucal, the principal product is the methyl α-D-mannoside.[26]

h. Furanoid Glycosides and Sugars from Carbonates

Haworth, Porter and Waine[27] utilized the stability of the carbonates to acids and instability to bases (see p. 177) for the preparation of the first crystalline furanosides and furanoses as illustrated below.

Methyl glucofuranoside 5,6-   Methyl α-glucofurano-
carbonate   side
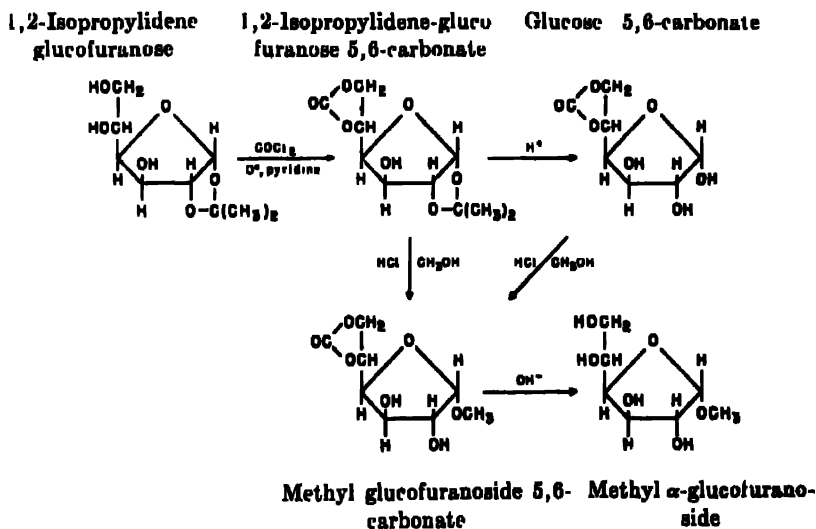
i. Enzymic Synthesis

In the Fischer method for the synthesis of glycosides, an alcohol and a sugar are condensed by the use of an acid as the catalyst. As has been shown in the work of Bourquelot and associates, enzymes may be utilized in place of the acid to catalyze the formation of glycosides. In recent times

[26] M. Bergmann and H. Schotte, *Ber.*, **54**, 1564 (1921).
[27] W N Haworth and C R Porter and A C Waine, *J. Chem. Soc.*, 2254 (1932).

this excellent work has been ignored to a considerable extent, but the method should be considered when it is desired to prepare glycosides for which the corresponding enzymes are known. For further discussion see Chapter XI.

j. TRANSGLYCOSIDATION

In nonaqueous solutions of hydrogen chloride, an equilibrium is established between the α- and β-glycosides, and the rate of rearrangement is proportional to the hydrogen chloride concentration. Under these conditions furanosides are converted to the pyranosides.[28] Sometimes the alkyl group is exchanged when the reaction takes place in a reactive solvent different from that which the glycoside yields on hydrolysis. In methyl alcohol containing hydrogen chloride, the ethyl α-D-glucoside is transformed to the methyl α-D-glucoside; the methyl and benzyl α-fructofuranosides yield the benzyl β-fructopyranoside when dissolved in benzyl alcohol under similar conditions.[29]

Under acetylation conditions with an acid catalyst (employing acetic anhydride, acetic acid and sulfuric acid) the acetylated methyl pyranosides are transformed to acetylated sugars of the pyranose structure, but the furanosides under the same conditions are changed to open-chain acetates or to ring acetates of possible furanose structure.[30] The methyl triacetyl-arabinopyranosides behave differently, possibly because the ring is formed through a primary alcoholic group. Thus 0.16 percent sulfuric acid (or zinc chloride) dissolved in acetic anhydride and glacial acetic acid brings about an equilibrium between a small amount of ring acetate and the penta-acetylarabinose methyl hemiacetals. More concentrated acid removes the methyl group and produces the peracetylated *aldehydo*-hexaacetyl-arabinose.

From the preparative standpoint, the transformation of acetylated β-glycosides to α-glycosides under the influence of titanium tetrachloride is of particular interest. It was shown by Pacsu that in chloroform solution (free of water and alcohol) titanium tetrachloride transforms the acetylated alkyl β-glycosides to their alpha isomers in high yield.[31]

$$n\text{-Hexyl tetraacetyl-}\beta\text{-glucoside} \xrightarrow[\text{CHCl}_3]{\text{TiCl}_4} n \text{ Hexyl tetraacetyl-}\alpha \text{ glucoside}$$
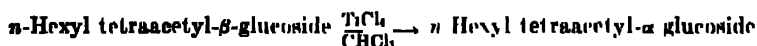
For the acetylated benzyl glucosides, the equilibrium mixture consists of about 90 percent of the alpha isomer.[32] It is of interest that under these

[28] J. W. Green and E. Pacsu, *J. Am. Chem. Soc.*, **59**, 1205 (1937)

[29] C. B. Purves and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 1170 (1937).

[30] E. Montgomery, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 1124 (1937).

[31] E. Pacsu, *J. Am. Chem. Soc.*, **58**, 2563, 2568, 2571 (1936).

[32] E. Piel and C. B. Purves, *J. Am. Chem. Soc.*, **61**, 2978 (1939).

conditions, the methyl β-cellobioside heptaacetate gives the alpha isomer. Since two beta glucosidic linkages occur in the original substance, either might be involved. If the configuration of the disaccharide linkage were changed, a derivative of maltose would be expected. Apparently, if it occurs, the transformation to a maltose derivative is only a minor reaction since the main product is the α-cellobioside. Fructosides exhibit either no reaction or the alkoxyl group is replaced by a halogen atom.[33] Aromatic glycosides under these conditions remain unaffected. Phenyl β-glycoside acetates, however, are convertible to the alpha forms on heating with zinc chloride in phenol.[34]

B. Properties of Glycosides. The glycosides are water-soluble substances except when the hydrocarbon aglycon becomes large enough to dominate the physical behavior of the compound. In the *n*-alkyl β-glucoside series, the glucosides become quite difficultly soluble in water when the aglycon has more than nine carbon atoms. The higher members of the *n*-alkyl series of glucosides are surface active and form liquid crystals at the melting point.[35]

The solubility of the surface-active materials in water is improved by treating the glycosides with alkylene oxides (*e.g.*, ethylene oxide) in the presence of catalysts such as sodium hydroxide or an amine.[36]

The increased solubility of glycosides as compared with the free aglycon has been utilized to enhance the effect of many pharmaceutical substances, the glycosides of 2-alkyl-1,4-naphthohydroquinone (an antihemorrhagic agent) provide an example.[37]

a. STABILITY TO ALKALINE HYDROLYSIS

It is usually considered that the glycosidic linkage is stable to the action of alkalies and consequently that glycosides exhibit no reducing action on Fehling solution. Nevertheless, some reduce Fehling solution. The first alkali-sensitive glycoside reported is apparently the 2,4,6-tribromophenyl β-glucoside of Fischer and Strauss.[38a]

Alkyl glycosides usually are more stable than aryl glycosides to the action of alkalies. However, when sulfonic ester or nitro groups are introduced in positions beta to the glucosidic linkage, the glucosides reduce Fehling

[33] E. Pacsu and F. B. Cramer, *J. Am. Chem. Soc.*, **59**, 1059 (1937)

[34] E. Montgomery, N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 690 (1942).

[35] C. R. Noller and W. Rockwell, *J. Am. Chem. Soc.*, **60**, 2076 (1938); W. W. Pigman and N. K. Richtmyer, *ibid.*, **64**, 369 (1942).

[36] French Patent 838,863, March 17, 1939; German Patent 715,543, Nov 27, 1941 ·

[37] B. Riegel and P. G. Smith, U. S. Patent 2,336,890, Dec. 14, 1913

[38] a. E. Fischer and H. Strauss, *Ber.*, **45**, 2167 (1912), b. See discussion by H. Gehman, L. C. Kreider and W. L. Evans, *J. Am. Chem. Soc.*, **58**, 2388 (1936)

solution.[39] In the series: X—CH$_2$—(CH$_2$)$_n$—CH$_2$—O—Gl (X = SO$_3$C$_2$H$_5$
or NO$_2$; Gl = glucosyl) the glucosides are reducing only when n = 0.
Several oligosaccharides exhibit alkali sensitivity.[39b, 40] Two such oligo-

                                          OGl
                                           |
saccharides are turanose: CH$_2$OH—(CHOH)$_3$—CH- CO—CH$_2$OH (3-
glucosyl-fructose) and dihydroxyacetone glucoside: CH$_2$OH—CO—CH$_2$-
O -Gl. These compounds and the phenyl glucosides have a common struc-
                              | |
tural unit, --C=C—OGl, which in the case of the oligosaccharides is the
enol-isomer of the above structures. As noted by Evans and associates,
such a structure seems to be responsible for the sensitivity to alkalies
The resemblance of this structure to that of the alkali-saponifiable esters
                        |
should be noted: O=C—OGl. When the double bond is removed farther
from the glycosidic linkage, as in allyl glucoside (CH$_2$=CH—CH$_2$O- Gl),
the lability in the presence of hydroxyl ions is lost. However, such a struc
ture does not explain the pronounced sensitizing action of NO$_2$ and –SO$_3$R
groups in certain positions as noted above.

The phenyl glucosides such as the tribromophenyl and nitrophenyl gluco-
sides exhibit sensitivity to hydroxyl ions. The aglycons (substituted
phenols) of these glycosides are stronger acids than phenol, and there may
be a correlation between the alkali sensitivity of the glycosides and the
acidities of the aglycons.[41] It might be considered that these substances
are more closely related to the alkali-hydrolyzable esters than to the stable
alkyl glycosides.

The nature of the hydrolytic products has not been sufficiently investi
gated, and it is usually assumed that the products are the same as those
produced by the action of acids. However, phenyl β-glycosides when heated
with aqueous barium hydroxide are converted to 1,6-anhydro sugars of
the levoglucosan type. The alpha isomers are only slowly affected by the
treatment and may be recovered largely unchanged. (See Glycosans.)
On the other hand, a double bond may be created in the aglycon. Thus,
picrocrocin (the bitter glucosidic principle of saffron) gives safranal on
treatment with alkali, although enzymic hydrolysis yields the expected
products.[42]

[39] B. Helferich and M. Hase, *Ann.*, **554**, 261 (1943); B. Helferich and H. Schnorr,
ibid., **547**, 201 (1941).

[40] H. S. Isbell, *J. Research Natl. Bur. Standards*, **26**, 35 (1941)

[41] J. H. Fisher, W. L. Hawkins and H. Hibbert, *J. Am. Chem. Soc.* **63**, 3031 (1941)

[42] R Kuhn and I Löw, *Ber.*, **74**, 219 (1941).
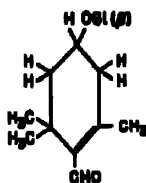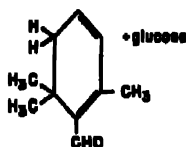
Picrocrocin   Safranal

Bromallyl β-glucoside has no action on Fehling solution but HBr is removed with the formation of propinyl β-glucoside (also nonreducing).

$$CH_2=CBr - CH_2-- OGl \xrightarrow{OH} CH\equiv C—CH_2---OGl$$

In common with acetylene, the glucoside reacts with copper salts. When it is present with a reducing substance, no reduction of Fehling solution is observed since a soluble salt is formed by the copper salts and the glucoside [4]

b. HYDROGENATION

The aromatic (phenyl) and benzyl glycosides are split by hydrogen with the aid of platinum catalysts (in the presence of hydrogen ions at room temperature and atmospheric pressure) to a hydrocarbon and sugar.[44][45]

$$Benzyl\ \beta\text{-}D\ glucoside\ \xrightarrow[H_2(H^+)]{Pt} Toluene + glucose$$

Hydrogenation with palladium catalyst under similar conditions proceeds differently and only benzyl β-glucoside is cleaved. For aromatic glucosides, this catalyst acts usually by hydrogenating the benzene ring, and the resulting cyclohexyl β-glucosides are not further affected. The use of palladium provides a method for the conversion of aromatic to cyclohexyl glucosides.[44] By the use of the palladium catalyst the following transformations are carried out: phenyl to cyclohexyl β-glucoside, and phenylpropyl to 3-cyclohexylpropyl β-glucoside (but salicin to o-cresyl β-glucoside). Catalytic hydrogenation has also been employed for the preparation of gentiobiose from amygdalin [46] Because of the great reactivity of the ethylenic linkage, this linkage may be preferentially hydrogenated even when an aromatic ring is present.[47] The cleavage of aromatic glycosides by the platinum catalyst is in contrast to the lack of reactivity of the alkyl glycosides. As

[43] B Helferich and J. Werner, *Ber*, **76**, 592 (1943)

[44] N. K Richtmyer, *J Am Chem. Soc.*, **56**, 1633 (1934).

[45] K. Freudenberg *et al.*, *Ber*, **61**, 1739, 1754 (1928).

[46] M. Bergmann and W Freudenberg, *Ber*, **62**, 2785 (1929)

[47] N K. Richtmyer and R M Hann, *J. Am. Chem. Soc.*, **57**, 227 (1935)

mentioned elsewhere, the reductive cleavage of the N-glycosides is ascribed to an equilibrium with the Schiff base $(R-N=CH-(CHOH)_4-CH_2OH)$, the reactive isomer (see Chapt. IX). One of the resonating forms of the phenols has a structure (II) analogous to that of the Schiff base and may be the reducible isomer. The existence of such structures only in aryl glycosides may explain the difference in the ease of hydrogenation of the alkyl and aryl glucosides as well as the greater acid stability of the alkyl glycosides.

(I)   (II)
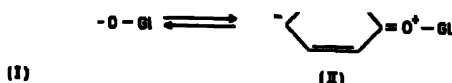
c. Ease of Hydrolysis by Acids

The rates of hydrolysis of many glycosides have been measured and provide excellent data for investigations of the influence of structural and configurational changes on the stability of the glycosidic linkage. Such comparisons can only be made in a qualitative fashion since the activation energies differ somewhat for the various glycosides and comparisons made at one temperature may not always agree with those at another temperature.

Effects of Variations in the Aglycon Structures of α- and β-glucosides. Moelwyn-Hughes gives the following data for the rates of acid hydrolysis at unit hydrogen-ion activity and at 60°C for several α- and β-glucosides (Table I).

As a rule, the glucosides with aliphatic aglycon groups (methyl and mandelonitrile glucosides) are more resistant to acid hydrolysis than those with aromatic aglycon groups. Although there is a considerable difference in the activation energies for the various glucosides, this difference does not seem to be related entirely to the aromatic or aliphatic character of the aglycon group. (For additional data see Table X of Chapter XI). Data on the rates of hydrolysis of di- and oligo-saccharides are given elsewhere (Table II, Chapter X).

The half-life and activation energies[48] for the hydrolysis of some glucosides and fructosides are compared in Table II. The methyl α-fructofuranoside is the most easily hydrolyzable glycoside in this series, but its rate and activation energy are not greatly different from those of the fructopyranosides.

Influence of Changes in the Configuration of the Carbons Composing the

48 L. J. Heidt and C. B. Purves, *J. Am. Chem. Soc.*, **66**, 1385 (1944)

Pyranose Ring. The methyl glycosides of many sugars have been prepared and their ease of acid hydrolysis studied.[49, 50] Some of the results are summarized in Tables III and IV.

<div align="center">

TABLE I

Acid Hydrolysis of Glucosides (60°C.)

</div>

Glucoside	k/a_{H^+} (sec^{-1}) $\times 10^6$	Activation Energy (cal./mole)
Methyl α-glucoside	1.46	38,190
Methyl β-glucoside	3.86	33,730
Mandelonitrile β-glucoside	1.07	34,040
Saligenin β-glucoside	18.0	31,630
Hydroquinone β-glucoside	43.4	30,760
Phloridzin	116	22,920
Methyl tetramethyl-α-glucoside	4.09	19,840

* E. A. Moelwyn-Hughes, *Trans. Faraday Soc.*, **25**, 503 (1929). It should be noted that, contrary to the usual custom of carbohydrate chemists of using decimal logarithms and minutes as units for calculating reaction constants, Moelwyn-Hughes uses natural logarithms and seconds, as is customary for physical chemists.

<div align="center">

TABLE II

Kinetics of the Hydrolysis of Glucosides and Fructosides

(0.05 N HCl at 60°C.)

</div>

Glycoside	Half-life (min.)	Activation Energy (cal./mole)
Methyl α-glucopyranoside	207,000	34,780
" β- "	104,000	33,460
Benzyl α- "	116,000	34,130
" β- "	69,700	31,460
Phenyl α- "	3,150	31,120
" β- "	11,500	32,200
Methyl α-fructopyranoside	6.2	27,790
" β- "	12.8	29,420
Benzyl β- "	7.4	27,780
Methyl α-fructofuranoside	2.2	26,950

As a first approximation, it is possible to study[49] the effect of variations in the configuration of the individual carbon atoms composing the pyranoside ring by comparing the rates of hydrolysis of substances which differ only in the configuration of a single carbon as is done in Table V. For

[49] H. S. Isbell and H. L. Frush, *J. Research Natl. Bur. Standards*, **24**, 125 (1940).

[50] C. N. Riiber and N. A. Sørensen, *Det. Kgl. Norske Videnskab. Selskabs Skrifter*, No. 1 (1938).

most glycosides, the beta isomers are more easily hydrolyzed than the alpha isomers, but the gulosides (and the corresponding heptosides of the

TABLE III

Velocity Constants Reported by Isbell and Frush[a]

Substance	0 03 N HCl at 98°C	0 5 N HCl at 75°C
Methyl α D lyxopyranoside	0 0037l	0 00286
Methyl β-D lyxopyranoside	0135	
Methyl α-D-mannopyranoside	00069	000471
Methyl β D mannopyranoside	00107	00113
Methyl α D-gulopyranoside	0125	0115
Methyl β D-gulopyranoside	00576	00377
Methyl α D gluco D *gulo*-heptopyranoside	00486	00417
Methyl β D-gluco-D *gulo* heptopyranoside	00219	00132
Methyl α-D-gala-L-*manno*-heptopyranoside	000381	
Methyl β-D gala-L-*manno* heptopyranoside	000860	
Methyl α-D-gala-L-*gluco*-heptopyranoside	000166	

* The velocity constants were calculated from the equation for a first order reaction and are expressed in Briggs logarithms and in minutes

TABLE IV

Velocity Constants Reported by Riiber and Sørensen[b]

Substance	Velocity constants*	
	0 01 N HCl at 100°C	0 5 N HCl at 75°C
Methyl α D glucopyranoside	0 000066	0 000198
Methyl β D-glucopyranoside	000137	000379
Methyl α-D-xylopyranoside	—	00090
Methyl β D xylopyranoside	—	0018
Methyl α-D-galactopyranoside	—	00104
Methyl β-D-galactopyranoside	—	00183
Methyl α L-arabinopyranoside	—	0018
Methyl β-L-arabinopyranoside	—	0026
Methyl α-L-rhamnopyranoside	00055	-
Methyl β-L rhamnopyranoside	00125	—
Methyl α-D-mannopyranoside	000137	
Methyl β-D-mannopyranoside	00021	

* The velocity constants were calculated from the equation for a first-order reaction and are expressed in Briggs logarithms and in minutes

gulose type) provide a marked exception. It will also be seen that the isomeric alpha and beta glycosides of the same sugar usually differ less in rate of hydrolysis than do the glycosides of the separate sugars The ratios given in Table V indicate that every asymmetric center in the pyranose

ring exerts an effect on the rate of acid hydrolysis The glycosides which have *trans* configurations for carbons 1 and 3 are hydrolyzed more slowly than the corresponding modifications which have the *cis* configuration

TABLE V

Effect of Configuration on Rate of Hydrolysis of the Hexopyranosides

(0 5 N HCl at 75°C)

Substances Differ at	Substances	$k k$
Carbon 1	Methyl β-D glucoside/methyl α-D glucoside	1 91
	Methyl β D mannoside/methyl α D mannoside	2 40
	Methyl β D galactoside/methyl α D galactoside	1 76
	Methyl β D guloside/methyl α D guloside	0 33
Carbon 2	Methyl α D mannoside/methyl α D glucoside	2 48
	Methyl β D mannoside/methyl β D glucoside	2 96
Carbon 3	Methyl α D guloside/methyl α D galactoside	11 06
	Methyl β D guloside/methyl β D galactoside	2 06
Carbon 4	Methyl α D galactoside/methyl α D glucoside	5 75
	Methyl β D galactoside/methyl β D glucoside	4 83
Carbon 5	Methyl α L guloside*/methyl β D mannoside	10 16
	Methyl β L guloside*/methyl α D mannoside	8 00

* Measurement made on enantiomorph

TABLE VI

Relative Ease of Acid Hydrolysis of Pentosides, Hexosides and Heptosides of Related Configuration

Group A	Mannoside Series		Glucoside Series		Galactoside Series	
	α	β	α	β	α	β
H	6 07	8 08	1 55	4 75	1 73	1 42
CH₂OH	1	1	1	1	1	1
CH₃	4 01	5 95	–	–	–	–
CHOH CH₂OH	0 55	0 51	0 50	–	–	–

This effect of the hydroxyl on carbon 3 possibly is to be ascribed to its proximity to the ring oxygen.

A comparison[49] of the ease of acid hydrolysis of homomorphous pentosides, hexosides and heptosides is made in Table VI. In each case, the ease

of hydrolysis relative to the hexoside is given. It will be seen from the table that there is invariably a decrease in the ease of hydrolysis of configurationally related series as the number of carbon atoms in the sugar chain increases. Even a small difference in the groups attached to carbon 5 such as that between mannose and rhamnose, which differ only in having a CH_2OH or a CH_3 attached to carbon 5, results in a conspicuous effect on the rate of acid hydrolysis.

The acid hydrolysis of methyl 2-desoxy-α-glucoside and of methyl 2-desoxy-α,β-cellobioside proceeds about 500 times more rapidly than the corresponding glucoside and cellobioside.[51] The nature of the groups at-

TABLE VII

Comparison of Ease of Hydrolysis of Pyranosides and Furanosides

(τ = 95-100°C.; 0 01 N HCl, k in minutes and Briggs logarithms)

Pyranosides		Furanosides	
Glycoside	$k \times 10^5$	Glycoside	$k \times 10^5$
Methyl α D glucoside	25	Methyl α D glucoside	4500
Methyl β D glucoside	30	Sucrose	5000
Methyl α D mannoside	10	Octamethylsucrose	1000
Methyl α D-galactoside	23	Ethyl β D glucoside	5300
Methyl tetramethyl α D glucoside	4	Ethyl tetramethyl β D glucoside	1400
Methyl tetramethyl β D glucoside	10		
Methyl tetramethyl α D mannoside	4	Methyl α D mannoside	1500
Methyl tetramethyl α D galactoside	4	Methyl tetramethyl α-manno side	250

tached to carbon 2, as well as those attached to carbon 5, exerts a pronounced influence on the rate of hydrolysis.

The marked effect of structural changes on the stability of the glycosidic linkage is demonstrated by a comparison of the ease of hydrolysis of pyranosides and furanosides. As shown in Table VII, the furanosides are hydrolyzed from 50 to 200 times more easily than the corresponding pyranosides.[52] This difference in the ease of acid hydrolysis of pyranosides and furanosides is sometimes used for the determination of the ring type present in oligosaccharides and glycosides. Such differences are usually encountered but exceptions are known. Thus, the methyl and benzyl β-

[51] M. Bergmann, H. Schotte and W. Lechinsky, *Ber.*, **55**, 158 (1922)
[52] W. N. Haworth, *Ber.*, **65** A, 50 (1932).

fructopyranosides are hydrolyzed at about the same rate as sucrose (a fructofuranoside) and only 10 to 13 times more slowly than the methyl and benzyl α-fructofuranosides[53] (see also Table II).

C. Determination of the Structures of Glycosides. Methylation Method. In order to illustrate the methylation method, the structures of the four methyl glucosides will be considered. Fischer was able to separate two crystalline methyl glucosides from the products obtained by the reaction of glucose and methyl alcohol in the presence of hydrogen chloride and to show that the mother liquors consisted of an amorphous labile isomer which he called γ-methyl glucoside.[54] This sirupy product was later demonstrated[55] to be a mixture of several isomers which exhibit a marked difference from the crystalline isomers in the case of condensation with acetone. (For a description of methylation methods, see Chapt. VIII).

a. PYRANOID STRUCTURE OF FISCHER'S CRYSTALLINE METHYL GLYCOSIDES

The crystalline methyl glucosides of Fischer, methylated by Purdie and Irvine, give two methyl tetramethylglucosides. On acid hydrolysis, both yield the same crystalline tetramethylglucose. This tetramethylglucose (I) is oxidized by nitric acid to inactive trimethylxylaric acid (*xylo*-trimethoxyglutaric acid) (II) and dimethyl-L-threaric acid (L(dextro)-tartaric acid) which were isolated from the oxidation mixture as their diamides.[56] The diamides had been previously characterized by Purdie and Irvine. The configuration of the trimethoxyglutaric acid produced proves that it represents the upper five carbons of the tetramethylglucose, and the position of the methyl groups on carbons 2,3 and 4 eliminates these positions for the ring connection. Since the original methyl glucosides (III) are oxidized to uronic acids[57] retaining the glycosidic structure (oxidation at carbon 6), the ring cannot involve the primary hydroxyl group at carbon 6, and must be of the pyranose type.

Tetramethylglucose   Trimethylxylaric acid (*xylo*-Trimeth-
                                               oxyglutaric acid)

(I)   (II)
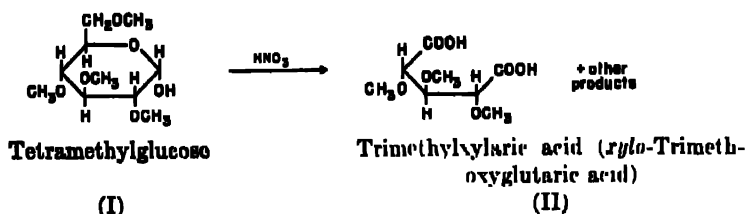
[53] C. B. Purves and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 1170 (1937).

[54] E. Fischer, *Ber.*, **47**, 1980 (1914).

[55] J. C. Irvine, A. W. Fyfe and T. P. Hogg, *J. Chem. Soc.*, 524 (1915).

[56] E. L. Hirst, *J. Chem. Soc.*, 350 (1926).

[57] K. Smolenski, *Chem. Abst.*, **19**, 41 (1925); K. Maurer and G. Drefahl, *Ber.*, **75**, 1489 (1942).

Methyl α-glucoside   Methyl α-glucuronide
(III)   (IV)
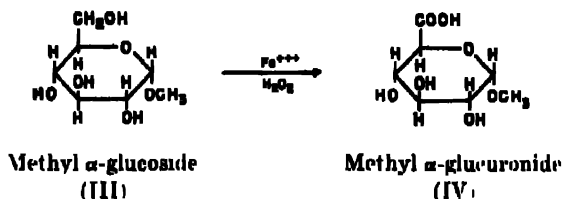
b. Furanoid Structure of the "γ" Methyl Glucosides

Methylation of Fischer's amorphous γ-methyl glucosides and hydrolysis by acids leads to a sirupy tetramethylglucose different from the crystalline product obtained from the crystalline methyl glucopyranosides.[55] Nitric acid oxidizes the sirupy tetramethylglucose to a mixture of dimethyl-L-threaric acid (dimethyl-L-tartaric acid) and oxalic acid.[54] (See accompanying formulas). These results are most easily interpreted as indicating the presence of a furanose ring with a connection between carbons 1 and 4 Oxidation of the linkage between carbons 4 and 5 would produce the products found, and the dimethyltartaric acid has the expected configuration Complete proof is given by periodic oxidation of the original glycosides (see below).

Methyl tetramethyl α   Tetramethylglucofuranose
glucofuranoside

Dimethyl L-threaric acid
(Dimethyl L-(dextro)-tar
taric acid)
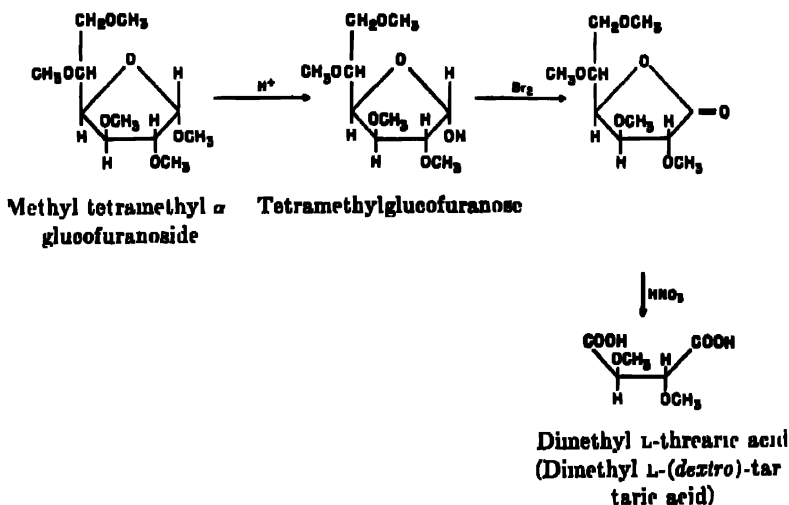
c. Pyranoid Nature of the Methyl Fructosides

D-Fructose yields two crystalline methyl fructosides.[59] [60] These may be

[55] W. N. Haworth, E. L. Hirst and E. J. Miller, *J. Chem. Soc.*, 2436 (1927).
[59] C. S. Hudson and D. H. Brauns, *J. Am. Chem. Soc*, **38**, 1216 (1916).
[60] H. H. Schlubach and G. A. Schröter, *Ber.*, **61**, 1216 (1928).

methylated and hydrolyzed to a crystalline tetramethylfructose[60, 61] which is oxidized by nitric acid to a monobasic acid. Acid permanganate oxidizes this product to crystalline 2,3,4-trimethyl-D-arabonic δ-lactone, whereas further oxidation yields trimethyl-D-arabaric (D-*arabo*-trimethoxyglutaric) acid. Since the configuration of the carbons 3,4 and 5 of fructose have the D-arabinose configuration, the methoxyls must be attached to these carbons in the tetramethylfructose, and the ring in all probability is between carbons 2 and 6 (pyranose type).[62]

Methyl tetramethyl-
fructopyranoside

Trimethyl-D-arabaric acid
(D *arabo*-Trimethoxy-
glutaric acid)

Trimethyl-D arabonic
δ-lactone

Trimethyl-D-arabinose
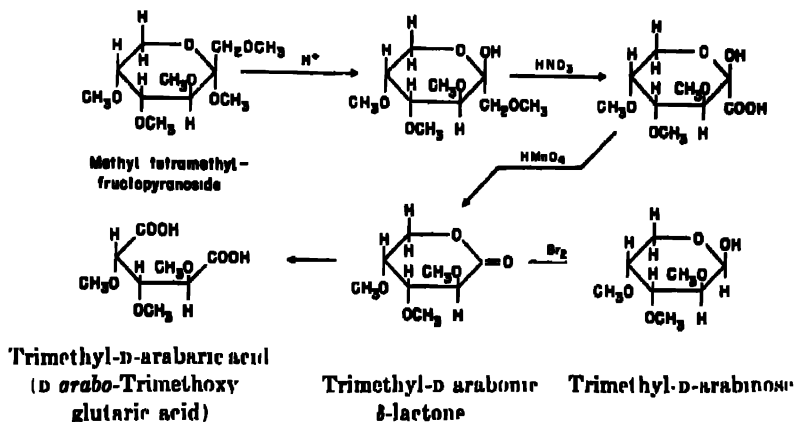
d. PERIODIC ACID OXIDATION*

After earlier work[63] had demonstrated that periodic acid and also barium hypobromite oxidize methyl glycosides by breaking the carbon chain, Jackson and Hudson[64] developed the periodic oxidation into an extremely convenient method for the determination of the ring structures of glycosides as well as for many other similar compounds. Since the reaction of the glycosides with periodic acid is quantitative, one simple method for the determination of the ring structure of a glycoside involves the measurement of the consumption of periodic acid. Two common types of reaction are illustrated in the following formulas.

The number of moles of periodic acid used by the most common types of rings are:

Four-carbon furanoside rings (n = 2), one mole of HIO_4.

* For additional discussion, see pages 46 and 328.
[61] T. Purdie and D. M. Paul, *J. Chem. Soc.*, **91**, 289 (1907).
[62] W. N. Haworth, E. L. Hirst and A. Learner, *J. Chem. Soc.*, 1040, 2432 (1927).
[63] H. Hérissey, P. Fleury and M. Joly, *J. pharm. chim.*, [8] **20**, 149 (1934).
[64] E. L. Jackson and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 994 (1937); **61**, 959 (1939); W. D. Maclay, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 1660 (1939), E L Jackson, *Organic Reactions*, **2**, 341 (1944)

Five-carbon pyranoside rings ($n = 3$), two moles of HIO_4.

Six-carbon heptanoside rings ($n = 4$), three moles of HIO_4.

$$
\begin{array}{c}
\overline{\left|\begin{array}{l} HCOCH_3 \\ | \\ (HCOH)_t \\ | \\ HC\text{----} \\ | \\ CH_2OH \end{array}\right.} O
\end{array}
\quad
\xrightarrow[\substack{n>1}]{(n-1)\,HIO_4}
\quad
\begin{array}{c}
\overline{\left|\begin{array}{l} HCOCH_3 \\ | \\ HCO \\ | \\ HCO \\ | \\ HC\text{----} \\ | \\ CH_2OH \end{array}\right.} O
\end{array}
\quad
\begin{array}{l}
H_2O \\ + \\ (n-1)\,HIO_3 \\ + \\ (n-2)\,HCOOH
\end{array}
$$

Hexopyranoside,
pentofuranoside or
heptoheptanoside
         (Four isomers)

$$
\begin{array}{c}
\overline{\left|\begin{array}{l} HCOCH_3 \\ | \\ (HCOH)_n \\ | \\ H_2C\text{----} \end{array}\right.} O
\end{array}
\quad
\xrightarrow[\substack{n>1}]{(n-1)HIO_4}
\quad
\begin{array}{c}
\overline{\left|\begin{array}{l} HCOCH_3 \\ | \\ HCO \\ | \\ HCO \\ | \\ H_2C\text{----} \end{array}\right.} O
\end{array}
\quad
\begin{array}{l}
H_2O \\ + \\ (n-1)HIO_3 \\ + \\ (n-2)HCOOH
\end{array}
$$

Pentopyranoside or
hexoheptanoside
         (Two isomers)

The nature of the ring is then easily determined by analysis of the quantity of periodic acid used in the reaction.

As illustrated above, the reaction product is a dialdehyde which in the first example has only two asymmetric carbons and which in the second example has only one asymmetric carbon. The asymmetric carbons remaining in the dialdehyde are those corresponding to carbon 1 and carbon 4 of the furanosides or carbon 5 of the pyranosides (the ring-forming carbon); but carbon 1 determines the α-β configuration, carbon 4 determines the D,L classification of the pentoses and carbon 5 the D,L classification of the hexoses. Hence, the hexopyranosides, the pentofuranosides and heptoheptanosides as a group yield only four stereoisomeric dialdehydes. These dialdehydes correspond to the α-D, the α-L, the β-D and the β-L glycosides and constitute two pairs of mirror images. The oxidation product of the pentopyranosides and of the hexoheptanosides has, however, only one asymmetric carbon which corresponds to the glycosidic carbon (carbon 1) of the original glycoside. These latter glycosides yield just two oxidation products which in turn are mirror images.

Dialdehydes obtained by periodic acid oxidation of certain methyl pyranosides and furanosides

```
CH₂OCH          HCOCH₃          CH₂OCH          HCOCH₃
  |               |               |               |
 HCO             HCO             HCO             HCO
       O               O     O         O
 HCO             HCO             HCO             HCO
  |               |               |               |
 HC              HC               CH              CH
  |               |               |               |
 CH₂OH           CH₂OH           CH₂OH           CH₂OH
   β               α               α               β
```

Hexopyranosides and pentofuranosides

```
CH₂OCH          HCOCH₃
  |               |
 HCO             HCO
       O               O
 HCO             HCO
  |               |
 H₂C             H₂C
 α-L, β-D        β-L, α-D
```
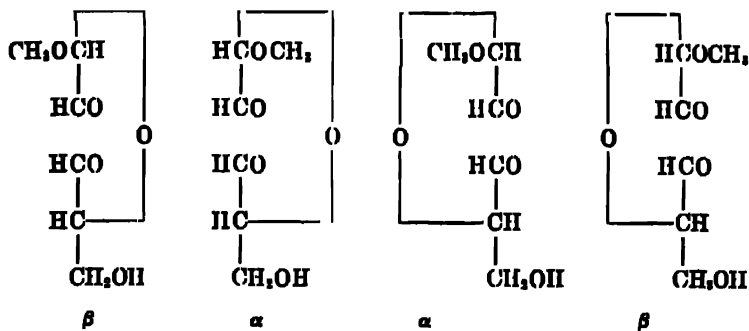
D and L,
Pentopyranosides and
hexoheptanosides
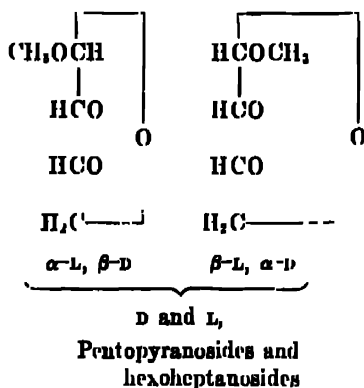
The identification of the reaction product provides valuable correlative evidence for the structure of the original methyl glycoside. The dialdehydes obtained as primary oxidation products are not well adapted to this purpose, but the corresponding acids obtained by bromine oxidation of the dialdehydes form easily identified salts. The diacids with two asymmetric carbons exhibit an interesting property; those from methyl β-D- and β-L-glycosides give nicely crystalline barium salts, and those from α-D- and α-L-glycosides characteristic strontium salts.

A convenient method for the identification of the reaction products requires observation of the change in the optical rotation during the oxidation. The various glycosides have different rotations, but as mentioned above a number of the asymmetric carbons lose their asymmetry and only six products (three pairs of mirror images) are produced by all of the hexopyranosides, the pentopyranosides and pentofuranosides. A measurement of the

specific rotation of the reaction product and a comparison of that obtained from glycosides of known structure fix the structure of the unknown Standard data for this purpose are illustrated by Figs. 4 and 5 which are taken from the work of Jackson and Hudson.

In the glycoside series, the periodic acid oxidation method has been applied only to certain methyl glycosides. For all of the compounds tested,
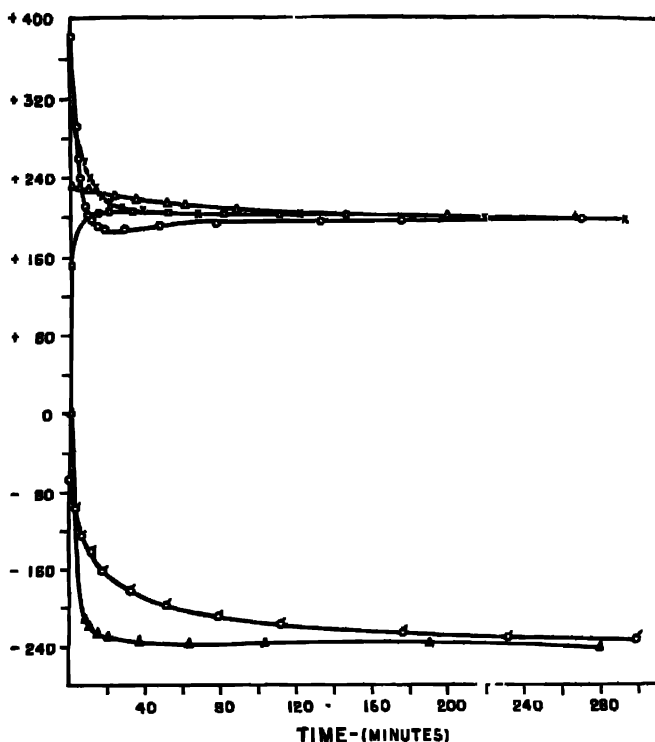
Fig 4 Rotatory changes during the oxidation of methyl hexopyranosides by periodic acid ⊙, methyl α-D galactoside, ✕, methyl α-D-glucoside; ⋀, methyl α-D-gulo-side, ▢ methyl α-D mannoside; ⊙, methyl β D-glucoside; ▲, methyl β-D-galacto side

(After Jackson and Hudson)

the method has given results agreeing with the configurations as deduced from the application of the optical rotation method. However, the methyl glycosides of some of the rare hexoses have not been prepared.

The Criegee lead tetraacetate oxidation of adjacent hydroxyl groups, when carried out in an organic solvent, also has been shown[65] to be very

[65] W S McClenahan and R C Hockett *J Am Chem Soc*, **60**, 2081 (1938); **61**, 1667 (1939), C Baer, J M Grosheints and H O L Fischer, *ibid*, **61** 2607 (1939), R Criegee, L Kraft and B Rank, *Ann*, **507**, 159 (1933)

similar to the periodic acid oxidation and may be used for the determination of the ring structure of glycosides and sugar derivatives. In aqueous solution, an additional mole of oxidant is required for each mole of formic acid produced since the latter is oxidized to carbon dioxide.[56]

In anhydrous solvents, lead tetraacetate is sensitive to *cis*, *trans* difference in adjacent hydroxyls. Aldohexofuranosides with *cis* hydroxyl groups
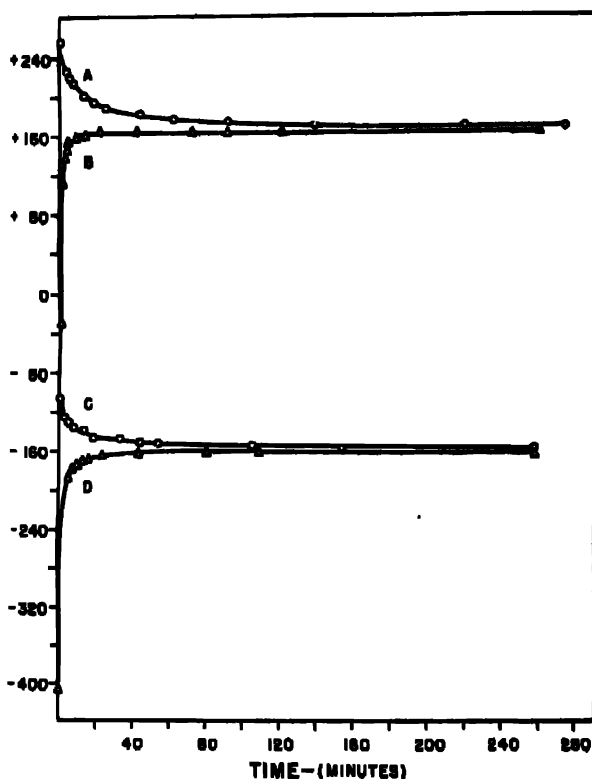
Fig. 5. Rotatory changes during the periodic oxidation of the methyl pentopyrano sides: A, methyl α-D-xylopyranoside; B, methyl α-D-arabinopyranoside; C, methyl β-D-xylopyranoside; D, methyl β-D-arabinopyranoside

(After Jackson and Hudson)

show, in acetic acid solution, a very rapid consumption of one mole of the oxidant followed by a slow consumption of a second mole. The corresponding *trans* compounds exhibit slower, more continuous consumption of more than two moles of oxidant.

Oxidation by atmospheric oxygen of solutions of methyl α-D-glucoside

in cuprammonium solution proceeds with cleavage of carbon bonds similar to that for periodic acid.[47]

2. Glycosans (Inner Glycosides)

Condensation of a hemiacetal hydroxyl with another hydroxyl group in the same molecule produces glycosans (inner glycosides), whereas condensation with a hydroxyl group provided by another molecule produces glycosides. Glycosan formation usually takes place indirectly or under conditions favoring dehydration (pyrolysis), but it is possible that small amounts of glycosans may be formed under conditions of glycosidation or during the hydrolysis of polysaccharides.

These compounds together with the inner ethers and some epoxy derivatives comprise a group known as anhydro carbohydrates.[68] Probably the best nomenclature is based on this type of name. Thus, levoglucosan is 1,6-anhydroglucopyranose. The nomenclature of disaccharides of this type (difructose anhydrides) is in need of clarification.

A. Preparation. Pyrolysis of sugars and polysaccharides under reduced pressure causes dehydration with the formation of anhydro sugars of the glycosan type. But near their melting points, glucose and other sugars yield 1,2-anhydro sugars[69] (ethylene-oxide rings), called "glucosane" by Gélis. The characterization of these latter products is doubtful. It is possible that they act as intermediates in the formation of the glycosans.
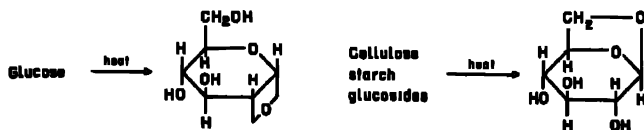
Dry distillation of cellulose, starch and glucosides[70] gives the isomeric 1,6-anhydro-β-D-glucopyranose also called D-glucosan <1,5> β <1,6>, levoglucosan and β-glucosan. The pyrolysis of lactose leads to the same 1,6-anhydroglucopyranose and, in addition, to the corresponding galactose derivative, 1,6-anhydro-β-D-galactopyranose (D-galactosan <1,5> β <1,6>). The polysaccharide agar gives small yields of the same 1,6-anhydrogalactose under similar conditions.[71] The direct pyrolysis of

[47] V. I. Ivanov and K. M. Sokova, *Compt rend acad sci U.R.S S.*, *42*, 175 (1944); *Chem Abst*, *39*, 2651 (1945)

[68] For a general discussion see. S. Peat, *Advances in Carbohydrate Chem.*, *2*, 37 (1946)

[69] A. Pictet and P. Castan, *Helv Chim. Acta*, *3*, 645 (1920); M. Cramer and E. H. Cox, *ibid*, *5*, 884 (1922)

[70] A Pictet and J Sarasin, *Helv Chim Acta*, *1*, 87 (1918); J C. Irvine and J. W. H Oldham, *J. Chem. Soc.*, 2729 (1925).

[71] R. M. Hann and C. S Hudson, *J. Am. Chem. Soc.*, *63*, 1484 (1941).

α-D-galactose produces the above isomer and 1,3-anhydro-β-D-galactopyranose (D-galactosan <1,5> β <1,3>). The 1,6-anhydro-β-D-mannopyranose (levomannosan or D-mannosan <1,5> β <1,6>) is obtained in a similar manner from ivory nuts of the ivory nut palm (*Phytecphas macrocarpa*). The anhydro sugars are found in the distillates from the pyrolysis of carbohydrates, and as shown by Hann and Hudson, the preparation of acetone derivatives increases the yields and facilitates the crystallization of many of the compounds.

Treatment of aromatic β-glucosides or thioglycosides with alkali at 100°C. provides an excellent method for the preparation of 1,6-anhydroglucopyranose (levoglucosan).[72] Even after long periods of heating, aromatic and alkyl α-glucosides and alkyl β-glucosides remain virtually unaffected. The corresponding 1,6-anhydrogalactopyranose is produced under similar conditions from both the phenyl α- and β-galactosides. Although the reaction takes place in a few hours for the β-isomer, several weeks are required for the α-isomer.
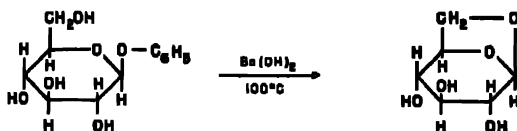
The mechanism of the reaction has been studied by McCloskey and Coleman.[73] It appears that a 1,2-anhydride may be an intermediate in the formation of levoglucosan because the reaction is blocked by the presence of a methoxyl group at carbon 2. On the other hand, the presence of methoxyl groups at carbons 3 and 4 does not prevent the reaction from proceeding normally with the production of levoglucosan.

The quaternary salt formed from tetraacetylgalactosyl bromide and trimethylamine yields 1,6-anhydro-β-galactopyranose on treatment with barium hydroxide.[74] During treatment of triacetyl-5-tritylribose with HBr, a trityl and an acetyl group are removed with the formation of 1,5-anhydro-2,3-diacetylribofuranose.[75] Acetylglycosyl halides with the hydroxyl of carbon 2 unsubstituted are transformed to 1,2-anhydro derivatives by ammonia in benzene or chloroform solution, and in this manner the 1,2-anhydro-3,4,6-triacetylglucose is prepared.[76]

[72] E. M. Montgomery, N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 3 (1943); *J. Org. Chem.*, **10**, 194 (1945); Tanret, *Compt. rend.*, **119**, 158 (1894).

[73] C. M. McCloskey and G. H. Coleman, *J. Org. Chem.*, **10**, 184 (1945).

[74] F. Micheel, *Ber.*, **62**, 687 (1920); P. Karrer and A. P. Smirnoff, *Helv. Chim. Acta*, **4**, 817 (1921).

[75] H. Bredereck, M. Köthnig and E. Berger, *Ber.*, **73**, 956 (1940).

[76] W. J. Hickinbottom, *J. Chem. Soc.*, 3140 (1928); P. Brigl, *Z. physiol. Chem.*, **122**, 245 (1922).

Sugars of the altrose type form anhydrides when heated with hydrochloric acid.[77] The altrose derivative has been identified as 1,6-anhydro-β-D-altropyranose. Sedoheptulose, which occurs in *Sedum spectabile*, and which has the altrose structure for the pyranose ring, forms an anhydride under similar conditions. According to evidence adduced by Hudson,[78] the compound has an unusual heptanose structure different from the anhydroaltrose and is to be considered as the 2,3-anhydro-sedoheptulo-heptanose Altrose and the anhydroaltrose when heated with acids attain an equilibrium consisting of 57 per cent of the anhydroaltrose and 43 per cent altrose.[79] For other hexose types, the equilibrium usually favors the formation of the sugar, and acid hydrolysis is utilized for the cleavage of anhydro rings.

A part of the unfermentable fraction obtained by refluxing concentrated fructose solutions consists of 1,2-anhydrofructopyranose (see below under Difructose anhydrides).

B. Difructose Anhydrides. An interesting group of fructose derivatives, obtained by the acid hydrolysis of inulin, have been studied in detail by Jackson, McDonald and Goergen.[80] These are anhydro derivatives of fructose disaccharides apparently formed during the hydrolytic reaction and not existing preformed in the inulin molecule. The structures of two of the three crystalline difructose anhydrides have been determined and are given in the following formulas
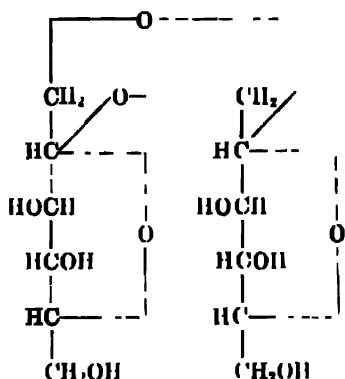
Difructose anhydride I (1,2′ 2 1′)   Difructose anhydride III (1,2′ 2,3′)
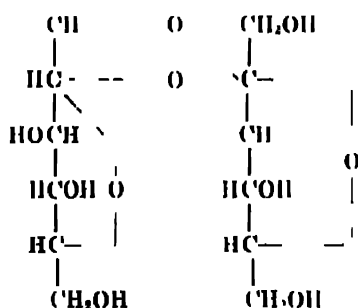
By treating fructose with concentrated hydrochloric acid at 0°C., Pictet

[77] N K Richtmyer and C S Hudson, J Am Chem Soc 62 961 (1940), G J Robertson and C I Griffith, J Chem Soc , 1196 (1935)

[78] C S Hudson J Am Chem Soc , 60, 1241 (1938)

[79] N K Richtmyer and C S Hudson, J Am Chem Soc , 61, 214 (1939)

[80] R J McDonald and R F Jackson, J Research Natl Bur Standards, 24, 181 (1940); F J McDonald, Advances in Carbohydrate Chem , 2 253 (1946), W N Haworth and H Streight, Helv Chim Acta, 15, 693 (1932)

and Chavan[81] produced two products which they called heterolevulosan and diheterolevulosan. On the basis of molecular weight determinations and other properties, the products are identified as a fructose anhydride and a difructose anhydride, respectively. A product similar to diheterolevulosan was shown by Schlubach and Behre[82] to have pyranose rings and 1,2′ and 2,1′ bridges. (The structure is similar to that for difructose anhydride I except for the presence of pyranose rings.) When concentrated aqueous solutions of fructose are refluxed, a portion of the material becomes unfermentable by yeasts. The unfermentable material consists of the reducing 1,2-anhydrofructopyranose and a difructose anhydride identical with that of Schlubach and Behre. The 1,2-anhydrofructopyranose is very labile and dimerizes to the difructose anhydride. These products appear to be present in the unfermentable ("glutose") fraction of cane molasses.[83]

Similar to the difructose anhydrides are the ethylene glycol glycoside anhydrides of glucose, galactose, and lactose, synthesized by Helferich and Werner[84] from 2′-chloroethyl tetraacetyl-β-glucoside, or the corresponding 2′-mesyloxyethyl β-glucoside, and alcoholic sodium hydroxide.
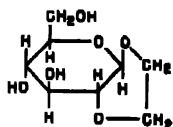
Ethylene glycol
β glucopyranoside 2 2′ anhydride
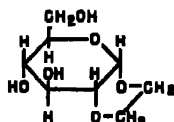
Ethylene glycol
α-glucopyranoside 2,2′ anhydride

The β-isomer is unattacked by 16 hours' boiling with normal sulfuric or hydrochloric acid, or by β-glucosidase. The corresponding lactose derivative, however, may be split by sulfuric acid, or the emulsins from sweet almond or lucerne, to glucose and ethylene glycol β-D-galactoside 2,2′-anhydride. The latter, like its glucose analog, is unattacked by acids or enzymes.

C. Structures. The determination of the structures of the glycosans, and anhydro sugars in general, is of particular importance because the preparatory methods give little direct information concerning the structures and because Walden inversions may take place. Methylation, followed by hydrolysis of the anhydro ring, gives partially methylated sugars which may be identified by nitric acid oxidation or by comparison with compounds of known structure.

The periodic acid oxidation is well adapted to the determination of the

[81] A. Pictet and J. Chavan, Helv. Chim. Acta, 9, 809 (1926).
[82] H. Schlubach and C. Behre, Ann., 508, 16 (1933)
[83] L. Sattler and F. W. Zerban, Ind. Eng. Chem., 37, 1133 (1945).
[84] B. Helferich and J. Werner, Ber., 75, 949, 1446 (1942)

rings present and the principle is the same as that described for the glycosides. For the purpose of illustrating the method, the arguments cited by Jackson and Hudson[85] for the structure of Pictet's levoglucosan are given. This substance upon oxidation by periodic acid or sodium metaperiodate produces one mole of formic acid and consumes two moles of the oxidant. This evidence demands that the compound have three contiguous hydroxyl groups and limits the possible structures to two types: 1,2-anhydrogluco-heptanose and 1,6-anhydroglucopyranose. The dialdehyde upon oxidation by bromine followed by acid hydrolysis gives strontium oxalate and strontium D-glycerate. As the formation of these products agrees only with the latter structure, levoglucosan is properly described as 1,6-anhydrogluco-pyranose. From the *cis* relationship of the hydroxyl of carbon 1 of β-glucose to the CH_2OH group, it is considered that the anhydro ring has the beta configuration since the connection is between carbon 1 and carbon 6. The crystalline strontium salt of the dibasic acid which was isolated serves as a reference compound for the determination of the structure of any anhydro hexose with similar structure (<1,5> β <1,6>), since the asymmetry of all carbon atoms except carbon 1 and carbon 5 is destroyed.

D. Reactions. The cleavage of anhydro rings of the glycosan type occurs in a manner similar to that for the inner ethers and epoxy types (see Chapter VIII). As a whole, the ease of cleavage of glycosan rings is intermediate between those of the epoxy and the five-membered types. Acetolysis of 1,6-anhydro-triacetylaltropyranose (triacetyl-altrosan) gives penta-acetylaltrose.[86]

3. Acetal and Mercaptal Derivatives of Acyclic Sugars

A. Mercaptals. In their reaction with alcohols in the presence of hydrogen chloride, the sugars differ from the simple aldehydes in forming mixed acetals (glycosides) rather than dialkyl acetals. The reaction with thio-alcohols (mercaptans) is similar for sugars and aliphatic aldehydes, however, and two thioalkyl groups are introduced to produce mercaptals (thioacetals).[87] The mercaptals are particularly valuable as intermediates for the preparation of open-chain (*aldehydo* and *keto*) derivatives and have been used by Wolfrom and by Brigl in their excellent work on the preparation of the *aldehydo* and *keto* derivatives of the sugars (see Chapter IV). Ordinarily, the mercaptals crystallize directly out of a mixture of the sugar, concentrated hydrochloric acid and mercaptan shaken at room temperature. The reaction is general for aldoses and aliphatic mercaptans but fails for

[85] E. L. Jackson and C. S. Hudson, *J. Am. Chem. Soc.*, **62**, 958 (1940).
[86] N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **63**, 1727 (1941).
[87] E. Fischer, *Ber.*, **27**, 673 (1894); W. T. Lawrence, *ibid.*, **29**, 547 (1896).

$$
\begin{array}{c}
\text{HC(SC}_2\text{H}_4)_2 \\
| \\
\text{HCOH} \\
| \\
\text{HOCH} \\
| \\
\text{HCOH} \\
| \\
\text{HCOH} \\
| \\
\text{H}_2\text{COH}
\end{array}
$$

D-Glucose $\quad -\dfrac{\text{C}_2\text{H}_5\text{SH}}{\text{HCl}}$

D-Glucose diethyl mercaptal
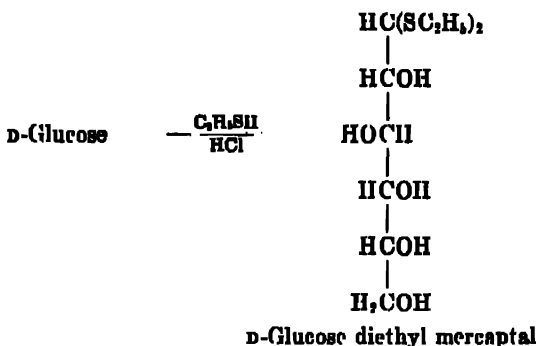
TABLE VIII

Properties of the Known Fully Acetylated Diethyl Mercaptals of Aldomonosaccharides

Fully acetylated diethyl mercaptal of	M. p	$[\alpha]_D^{20}$ (c < 5, CHCl₃)
	(°C)	
D-Arabinose	80	+30°
L-Arabinose	79–80	−30
D-Xylose	40–48	+13
D-Lyxose	36–37	+40.5
D-Glucose	45–47	+11
D-Galactose (trimorphous)	76.5–77	+11
	80.5–81	+11
	90.5–91	+11
6-Desoxy L galactose (L fucose)	99–100	+5
D Mannose	52 53	+32
6 Desoxy L mannose (L rhamnose)	59–61	−12
D-Gluco D gulo-heptose	99 100	−12
D Gala L gluco-heptose	105	+27
D-Gala-L-manno-heptose	145–146	+50
D-Manno D gala-heptose	77	−2.2
D Gala-L gala-octose	106	+30
Methyl D-galacturonate	112 5–113 5	+20.5
Ethyl D-galacturonate	80–81	+11

the ketoses and for the thiophenols. The fructose mercaptals may be obtained, however, by reaction of the acetylated or benzoylated *keto*-fructose with the mercaptan and a catalyst.[88] The *aldehydo*-acetylsugars add mercaptans to form hemimercaptals.[89]

As shown earlier in this chapter, the thioalkyl groups may be removed

[88] P. Brigl and R. Schinle, *Ber.*, 66, 325 (1933); M L Wolfrom and A. Thompson, *J. Am. Chem. Soc.*, 56, 880 (1934).

[89] M. L. Wolfrom, D Weisblat and A Hanze, *J Am. Chem. Soc.*, 62, 3246 (1940).

from the sugar mercaptals, and, according to the conditions employed, furanosides, pyranosides, thioglycosides or acetals are obtained

The stability of the mercaptals and their ease of preparation make them particularly important derivatives for the identification of the mono-saccharides Table VIII lists the properties of a number of these deriva-tives [90]

An interesting type of mercaptal, very stable to hydrolysis by acids, is that made from dithioglycol or 1,3-propanedithiol:

$$
\begin{array}{cc}
\mathrm{CH\,OH- -(CHOH)_4-CH} & \begin{array}{c} \mathrm{S-CH} \\ \diagup \\ \diagdown \\ \mathrm{S-CH_2} \end{array} \qquad , \qquad \mathrm{CH_2OH-(CHOH)_4- CH} & \begin{array}{c} \mathrm{S-CH_2} \\ \diagup \\ \diagdown \\ \mathrm{S-CH} \end{array} \qquad \mathrm{CH_2}
\end{array}
$$
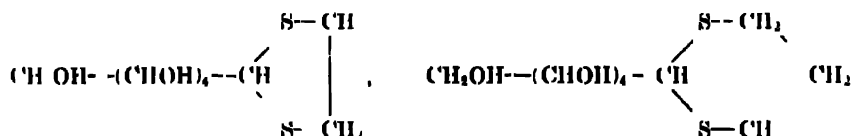
In addition to their use for the preparation of open-chain sugars, the sugar mercaptals are of value in the preparation of desoxyglykitols.[91] Mercaptals of aldoses yield 1-desoxyglykitols and of ketoses yield 2 desoxyglykitols upon reduction employing Raney nickel D-Galactose di-ethyl mercaptal gives L-fucitol, and D-fructose diethyl mercaptal gives 2-desoxy-D-sorbitol (as the pentaacetates)

$$
\begin{array}{ccc}
\mathrm{CH_2OH} & & \mathrm{CH_2OH} \\
| & & | \\
\mathrm{C(SR)_2} & \xrightarrow[\mathrm{H_2}]{\mathrm{Ni}} & \mathrm{CH_2} \\
| & & | \\
\mathrm{HOCH} & & \mathrm{HOCH} \\
|
\end{array}
$$
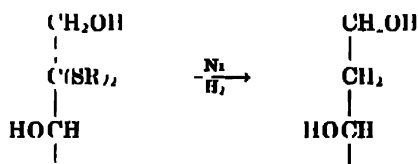
B. Acetals. Acetal derivatives of simple acyclic sugars (glycolaldehyde and glyceraldehyde) have long been known, but special reactions are required to prepare the acetals of the tetroses and higher sugars since the glycosides (mixed acetals) are preferably formed. Several methylated sugar dimethyl acetals have been reported,[92] but the first unsubstituted acetal in the sugar series is the D-arabinose dimethyl acetal of Montgomery, Hann and Hudson [93] The method used for the preparation (see formulas below) depends on the action of zinc chloride, under acetylating conditions, in rupturing the oxygen-carbon linkage of the pyranose ring of the methyl arabinosides. As intermediate products, the fully acetylated hemiacetal and the 1-chlorotetraacetylarabinose methyl hemiacetal are produced Deacetylation of the final product gives D-arabinose dimethyl acetal

[90] M L Wolfrom and J V Karabinos, *J Am Chem Soc*, 67, 500 (1945)

[91] M L Wolfrom and J V Karabinos, *J Am. Chem Soc*, 66, 909 (1944), J Bougault, E Cattelain and P Chabrier, *Bull soc chim*, [5] 5, 1699 (1938), [5] 7, 780 781 (1940)

[92] P A Levene and G Meyer *J Biol. Chem*, 74, 695 (1927)

[93] E Montgomery, R M Hann and C S Hudson, *J Am Chem Soc*, 59, 1124 (1937)

CH₂OCH   II
CH₂OCOAc

AcOCH   →(Ac)₂O ZnCl₂ AcOCH → AlCl₃

—C— O   —C—

Methyl D-arabino
pyranoside
triacetate

         II
         CH₂OCCl   II
CH₂OCOCH₃

         AcOCH → Ag₂O / CH₃OH   AcOCH

         —C—   —C—

Tetraacetyl-D
arabinose
dimethyl acetal
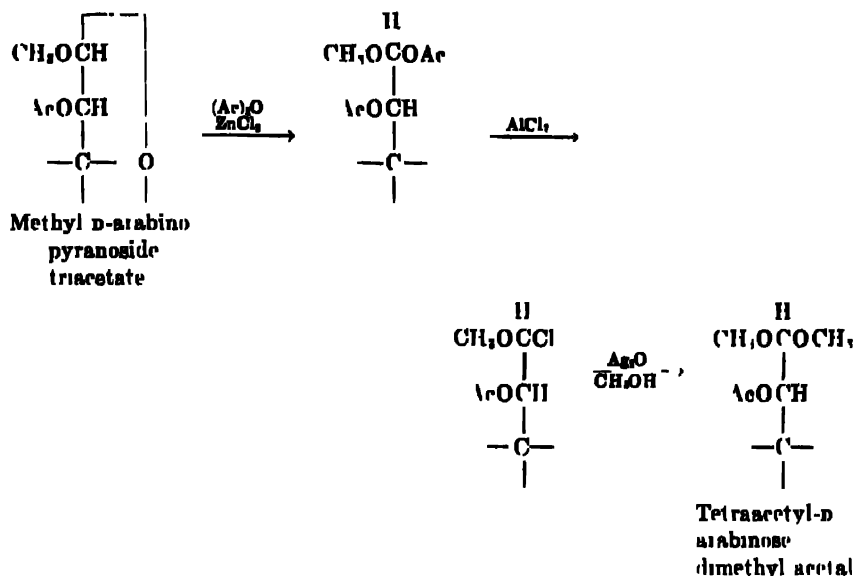
A more direct method for the preparation of sugar acetals depends on
the substitution of the thioalkyl radicals of mercaptals, for which ring
closure is blocked by the presence of substituent groups such as acetyl
groups [94] If ring closure is not prevented glycosides are usually formed

HC(SC₂H₅)₂   HC(OCH₃)   HC(OCH₃)₂

(CHOAc)₄ → CH₃OH (boil) CdCO₃ HgCl₂   (HCOAc)₄ → Ba(OC₂H₅)₂ (deacetylation)   (CHOH)₄

CH₂OAc   CH₂OAc   CH₂OH

Tetraacetyl
D-glucose
diethyl
mercaptal

D-Glucose
dimethyl acetal
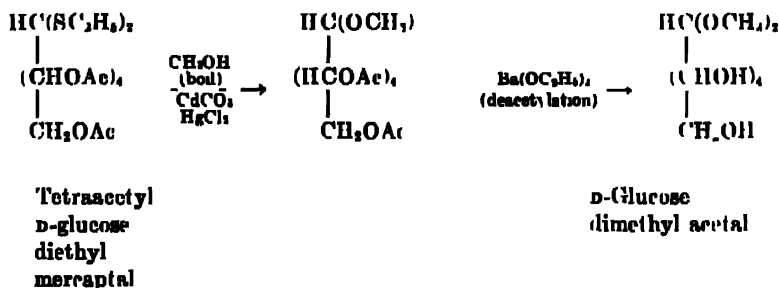
Fructose mercaptals and those of a few other sugars, particularly at low
temperatures, may form acetals by this method even when ring closure is
possible.[95]

Mixed acetal and thioacetal derivatives of the sugars are obtained from
the hemimercaptals.[96] These derivatives are the probable intermediates
in the conversion of mercaptals to furanosides (p 195)

[94] M L. Wolfrom and S Winsbrot, J Am Chem Soc 60, 851 (1938)
[95] E. Pacsu J Am Chem Soc, 61, 1671 (1939)
[96] M L. Wolfrom, D Weisblat and A Hanze J Am Chem Soc 66 2065 (1944), 62, 3246 (1940).

$$
\begin{array}{ccc}
\text{OAc} & \text{Cl} & \text{OCH}_3 \\
| & | & | \\
\text{HCSC}_2\text{H}_5 & \text{HCSC}_2\text{H}_5 & \text{HCSC}_2\text{H}_5 \\
| \quad \xrightarrow[\substack{\text{HCl} \\ 0-20°C}]{\text{ether}} & | \quad \xrightarrow[\text{Ag}_2\text{CO}_3]{\text{CH}_3\text{OH}} \xrightarrow[\substack{\text{(deacet-} \\ \text{ylation)}}]{\text{OH}^-} & | \\
(\text{CHOAc})_4 & (\text{CHOAc})_4 & (\text{CHOH})_4 \\
| & | & | \\
\text{CH}_2\text{OAc} & \text{CH}_2\text{OAc} & \text{CH}_2\text{OH}
\end{array}
$$
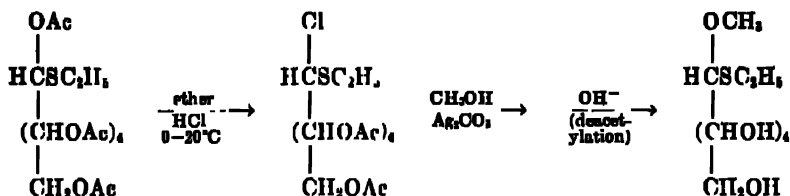
Hemiacetals are formed by direct addition of alcohols to many *aldehydo*-acetylsugars[97]; this reaction provides an explanation for the mutarotation of the parent substances in alcoholic solution. Two isomeric hemiacetals are possible for each sugar, for carbon 1 becomes asymmetric. The acetals are converted to the corresponding pyranosides in alcoholic hydrogen chloride (Fischer conditions for preparation of glycosides) and, hence, are in equilibrium with the glycosides, but the equilibrium favors the glycosides.

4. Reactions of Carbohydrates with Aldehydes and Ketones

Aldehydes react with alcohols to form acetals:

$$\text{RCHO} + \text{R'OH} \rightarrow \text{RCH(OR')}_2$$

Two hydroxyls of a carbohydrate molecule may react with an aldehyde with the formation of a cyclic acetal. These products are known as arylidene or alkylidene derivatives as, for example, benzylidene or ethylidene sugars. Acetone condenses similarly to give isopropylidene (acetone) derivatives. The first cyclic acetals of this type were prepared by Wurtz[98] who showed that ethylene glycol and acetaldehyde react when heated together and that one mole of water is lost. Mineral acids, zinc chloride, calcium chloride and copper sulfate greatly accelerate the reaction.[99]

$$
\begin{array}{c}
\text{CH}_2\text{OH} \\
| \quad + \quad \text{CH}_3\text{CHO} \quad \xrightarrow{\text{HCl}} \\
\text{CH}_2\text{OH}
\end{array}
\qquad
\begin{array}{c}
\text{CH}_2\!-\!\text{O} \\
 \diagdown \diagup \text{H} \\
| \qquad \text{C} \\
 \diagup \diagdown \\
\text{CH}_2\!-\!\text{O} \qquad \text{CH}_3
\end{array}
$$
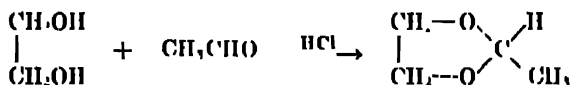
Other aldehydes (valeraldehyde, benzaldehyde, formaldehyde) condense with polyols to form cyclic acetals.[100] The analogous reaction of acetone with polyols and sugars to yield crystalline products was described by E. Fischer.[101]

[97] M. L. Wolfrom, M. Konigsberg and F. Moody, *J. Am. Chem. Soc.*, **62**, 2343 (1940); R. J. Dimler and K. P. Link, *ibid.*, **62**, 1216 (1940).

[98] A. Wurtz, *Ann.*, **120**, 328 (1861).

[99] A. Geunther, *Ann.*, **126**, 65 (1863); E. W. Adams and H. Adkins, *J. Am. Chem. Soc.*, **47**, 1358 (1925).

[100] For references to early work, see: E. Fischer, *Ber.*, **27**, 1521 (1894); B. Tollens, *ibid.*, **32**, 2585 (1899), H. Schiff, *Ann.*, **244**, 19 (1888).

[101] E. Fischer, *Ber.*, **28**, 1147 (1895); A. Speier, *ibid.*, **28**, 2531 (1895).

In ethylideneglycol, a five-membered ring is present, but in the acetal derivatives of trimethylene glycol a six-membered ring must be present. In polyols and sugars, these and other types of rings are possible. This subject was investigated for the lower polyols particularly by Hibbert and associates.

Hibbert's method depended upon studying the nature and amounts of the reaction products when alternate courses of reaction were possible. Thus, when equimolar quantities of acetaldehyde,[102] glycol and glycerol were allowed to react, the major product (1,3-ethylideneglycerol) had a six-membered ring and the minor product (ethylideneglycol) a five-membered ring.[103] Hence, a preference for the larger ring is indicated.

In a similar manner, the reaction of trimethylacetaldehyde with glycerol was studied.

$$ (CH_3)_3C-CHO \ + \ \begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \ \rightarrow \ (I) \ + \ (II) $$

(I)   (II)
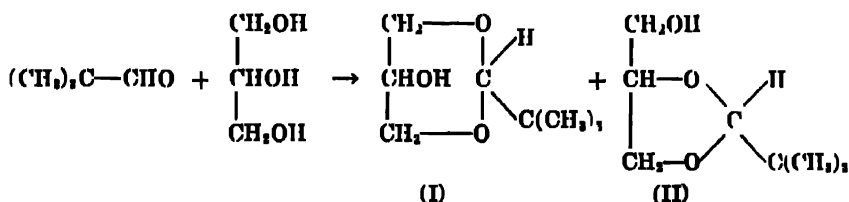
The distribution of the products between 6- and 5-membered rings (I and II) was in the ratio 2:3. The nature of the substitution was shown by methylation of the products, hydrolysis of the acetal residues, and identification of the monomethyl ethers.[104] In general, for glycerol, it was shown that such condensations reach a reversible equilibrium between the various possible isomers. Chloral and acetone form five-membered rings exclusively.

Ring shifts have been observed for the acetone derivatives of dulcitol when benzoylation was attempted. The D,L-2,3-5,6-diisopropylidene-galactitol is converted to 1,6-dibenzoyl-2,3-4,5-diisopropylidene-galactitol.[105]

It is probable that such condensations take place through the intermediate formation of an open-chain hemiacetal. In fact, the intermediate product was isolated in the case of chloral and ethylene glycol.[106]

$$ \begin{array}{c} CH_2OH \\ | \\ CH_2OH \end{array} \ + \ CCl_3CHO \ \rightarrow \ \begin{array}{c} CH_2O-CH(OH)-CCl_3 \\ | \\ CH_2OH \end{array} \ \rightarrow $$
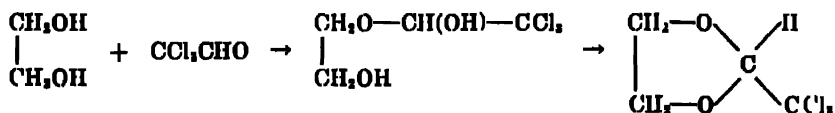
[102] Actually, acetylene with mercuric sulfate was used instead of acetaldehyde; see below under Ethylidene Derivatives.

[103] H. S. Hill and H. Hibbert, J. Am. Chem. Soc., 45, 3117 (1923).

[104] S. M. Trister and H. Hibbert, Can. J. Research, 14 B, 415 (1936).

[105] R. M. Hann, W. D. Maclay and C. S. Hudson, J. Am. Chem. Soc., 61, 2432 (1939)

[106] De Forcrand, Compt. rend., 108. 618 (1889). See also, H. Adkins and A. E. Broderick, J. Am. Chem. Soc., 50, 499 (1928).

The general types of ring found in acetal and ketal derivatives are the 6- and 5-membered dioxolane rings, respectively. Sometimes, 7-membered rings are formed, e.g., with trimethyleneglycol.

After their structures were established, these products became very important in the synthesis of partially substituted carbohydrates of known structure. Their use for this purpose arises from their stability under alkaline conditions and their ease of hydrolysis under mild conditions of acidity. Thus, free hydroxyl groups in acetonated sugars can be esterified or alkylated, and the blocking acetone groups subsequently can be removed by treatment with acid. Although benzylidene groups are relatively stable to acids, they may be removed easily by catalytic hydrogenation.

A. Methylene (Formal) Derivatives. Sugars, polyols and sugar acids react with formaldehyde in the presence of acids to yield mono- or di-methylene derivatives or, in the case of hexitols, trimethylene derivatives.[107] Crystalline monomethylene-glucose made by this method has a free reducing group. A sirupy dimethylene-glucose, made by melting glucose and trioxymethylene together, has no reducing properties.[108]

The structures of the methylene derivatives of the sugars have not been determined, but those of many of the polyol derivatives are known. In general, formaldehyde condenses most readily with *cis* secondary hydroxyl groups located β to one another. This preference for secondary hydroxyl groups is demonstrated by the ease with which acetolysis of methylene linkages at primary hydroxyl groups takes place. The difference in ease of acetolysis of methylene groups connected with primary from those with secondary alcoholic groups has been of considerable value in the elucidation of the structures of these compounds.[109] Thus the linkages at carbons 1 and 6 of 1,3-2,4-5,6-trimethylene-D-sorbitol are cleaved with sulfuric acid in acetic anhydride and acetic acid as shown in the formulas. The structure of the monomethylene-sorbitol is demonstrated by its oxidation with periodic acid to a monomethylene-xylose which is reduced to mono-methylene-xylitol. The latter must have the 2,4 structure with no neighboring pairs of hydroxyl groups, because it is not attacked by periodic acid.

B. Benzylidene Derivatives. Benzaldehyde reacts[110] with glucose and with methyl α- and β-glucopyranosides to form the 4,6-benzylidene derivatives. In the benzylidene and related compounds, the acetal carbon is

[107] M. Schulz and B Tollens, *Ann.*, **289**, 20 (1896); B. Tollens, *Ber.*, **32**, 2585 (1899)

[108] C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **22**, 159 (1903).

[109] A T Ness, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **66**, 665, 670 (1944)

[110] L Zervas, *Ber.*, **64**, 2289 (1931); J. C Irvine and J. P Scott, *J. Chem. Soc.*, **103**, 575 (1913); H Ohle and K Spencker, *Ber.*, **61**, 2387 (1928)

Trimethylene-
D-sorbitol

Monomethylene-   Monomethylene-
D-sorbitol   L-xylose
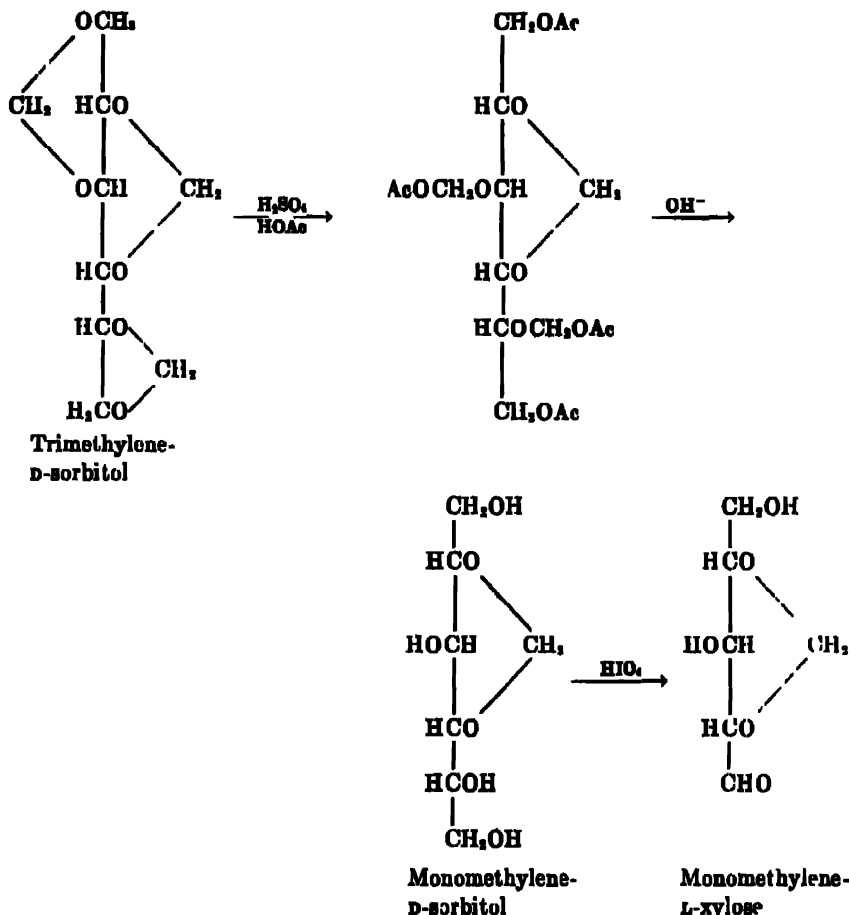
asymmetric and should give rise to two isomers, but the expected isomers have never been separated.

4,6-Benzylidene-D-glucose
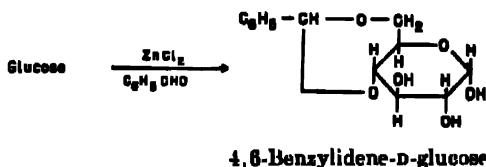
The monobenzylideneglucose, formed according to the reaction illustrated, reduces Fehling solution and forms a hydrazone and an osazone; therefore, it must have carbons 1 and 2 unsubstituted. The following sequence of reactions furnishes evidence to fix the structure of the substance as 4,6-benzylideneglucose.[111]

[111] K. Freudenberg, H. Toepffer and C. C. Andersen, Ber., 61, 1750 (1928)

Monobenzylideneglucose $\xrightarrow[\text{(one mole)}]{\overset{(CH_3)_2SO_4}{NaOH}}$

methyl benzylideneglucoside (I) $\xrightarrow[\text{(excess)}]{(CH_3)_2SO_4}$

methyl dimethylbenzylideneglucoside (II); 3-methylglucose $\xrightarrow[\text{CH}_3\text{OH}]{\text{HCl}}$

methyl 3-methylglucoside $\xrightarrow{C_6H_5CHO}$

methyl 3-methyl-benzylideneglucoside $\xrightarrow[\text{NaOH}]{(CH_3)_2SO_4}$

methyl dimethylbenzylideneglucoside (II).
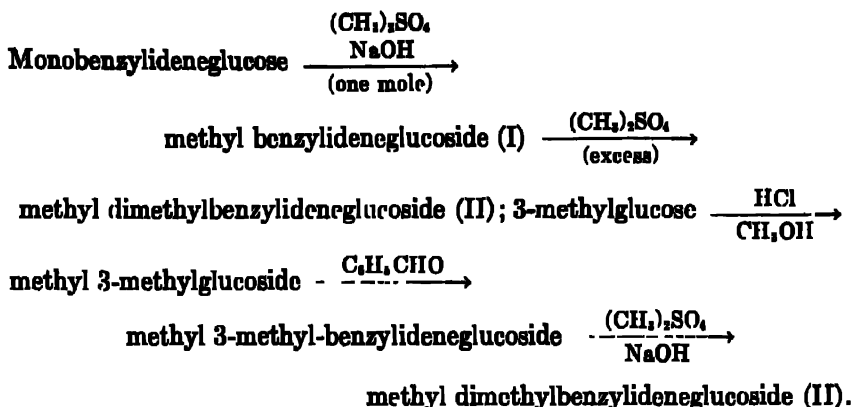
The latter compound (II) is the same as that (II) synthesized above. From this evidence, the original benzylideneglucose must have been unsubstituted at carbons 1, 2 and 3. Inasmuch as the benzylidene group may be removed from methyl benzylideneglucoside (I) (by catalytic reduction) with the formation of methyl β-glucopyranoside, the ring must be of the pyranose type. The benzylidene group must occupy positions 4 and 6, and the original compound is 4,6-benzylideneglucose.

The condensation of benzaldehyde (and probably other aldehydes) with sugars ordinarily does not produce furanose derivatives as so frequently happens in acetone-condensation reactions, discussed later. When the furanose ring is already present in the sugar derivative undergoing condensation, as in 1,2-isopropylidene-glucofuranose (monoacetoneglucose), the acetal ring formed is still of the six-membered type. For the example given, 1,2-isopropylidene-3,5-benzylideneglucofuranose is formed.[112] However, a second isomer with a five-membered ring is formed in substantial quantities (1,2-isopropylidene-5,6-benzylideneglucofuranose).[113]

C. Condensation with Acetaldehyde and Furfuraldehyde. Acetaldehyde condenses with glucose, in a manner analogous to that for benzaldehyde, to give 4,6-ethylidene-α-D-glucose (III).[114] With sorbitol, a triethylidene derivative is formed which probably has three five-atom rings.[115] The action of sulfuric acid on paraldehyde is a convenient source of acetaldehyde, but with methyl α-D-glucoside a product (IV) is formed (methyl 2,3-oxidodiethylidene-4,6-ethylidene-α-D-glucoside) which is unique in having a seven-membered ring formed from trans hydroxyl groups.[116]

[112] P. Brigl and H. Gruner, Ber., 65, 1428 (1932).

[113] P. A. Levene and A. L. Raymond, Ber., 66, 384 (1933).

[114] B. Helferich and H. Appel, Ber., 64, 1841 (1931); R. Sutra, Bull. soc. chim., [5] 9, 791 (1942); Chem. Abst., 38, 3257 (1944).

[115] H Appel, J. Chem. Soc, 425 (1935).

[116] H. Appel and W. N. Haworth, J. Chem. Soc., 793 (1938).
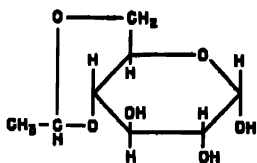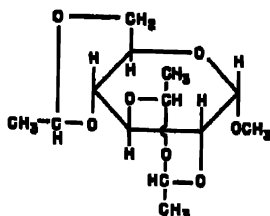
(III)   (IV)

Ethylidene derivatives of polyols have been made by treatment of a polyol with acetylene in the presence of sulfuric acid and mercuric sulfate.[117] Apparently the intermediate vinyl hydroxyethyl ether is formed which then condenses with a second hydroxyl group.
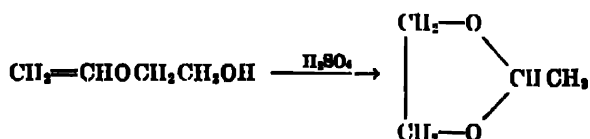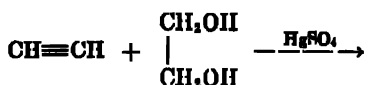
Proof for the mechanism is given by the ease with which the second reaction occurs when the vinyl ether, made by other methods, is allowed to react.[118]

Furfural reacts with methyl α-D-mannoside yielding mono- and di-furylidene derivatives[119] which have both five and six-membered rings as illustrated in the following formulas. With methyl α-glucoside a 4,6-mono derivative was obtained. The furylidene groups cannot be removed by catalytic hydrogenation as is the case for benzylidene groups.
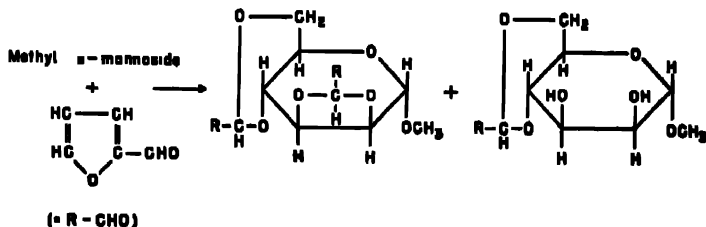
Cyclic acetals made by the reaction of furfural and glycol or glycerol have been suggested as plasticizers for lacquers. In the preparation of these

[117] German Patent 271,381 (1914); H. S. Hill and H. Hibbert, *J. Am. Chem. Soc.*, **45**, 3108 (1923).

[118] H. S. Hill and L. M. Pidgeon, *J. Am. Chem. Soc.*, **50**, 2718 (1928).

[119] H. Bredereck, *Ber.*, **68**, 777 (1935).

compounds, no catalyst is used, and the water is removed by azeotropic distillation with benzene.[120]

D. Isopropylidene (Acetone) Derivatives. The sugars and derivatives react with anhydrous acetone at room temperature in the presence of HCl, H_2SO_4, $ZnCl_2$, $CuSO_4$ or P_2O_5, and di- or mono-isopropylidene derivatives are formed.[121]
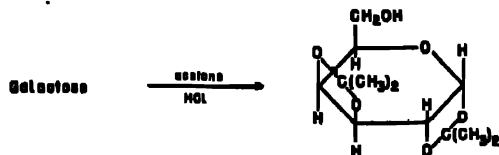
1,2-3,4-Diisopropylidenegalactose

In most instances, condensation takes place between the acetone and *cis* hydroxyl groups on contiguous carbon atoms in such a manner as to favor the introduction of two isopropylidene residues into the molecule.[122] For α-galactose, the hydroxyl groups in positions 1 and 2 are on the same side of the ring (*cis*), and those in positions 3 and 4 also have a *cis* relation although the latter pair is on the opposite side of the ring from that at carbons 1 and 2. One acetone molecule reacts with one of these *cis* pairs and a second with the other pair. However, α-glucopyranose has only one pair of *cis* hydroxyls (those at positions 1 and 2) and in order to make a second pair available, the furanose isomer reacts and forms 1,2-5,6-di-isopropylidene-D-glucofuranose.
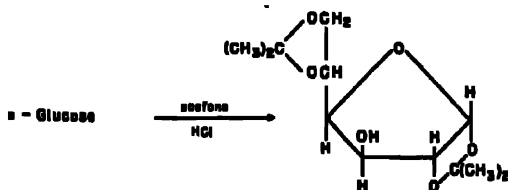
1,2-5,6-Diisopropylideneglucofuranose

Mannose also can provide two pairs of *cis* hydroxyls only in the furanose ring form, and the 2,3-5,6-diisopropylidene-D-mannofuranose is formed.[123]

[120] K. H. Hoover, U. S. Patent 1,931,309, Nov. 7, 1934

[121] E. Fischer and C. Rund, *Ber.*, **49**, 93 (1916); H. Ohle and I. Koller, *ibid.*, **57**, 1566 (1924); D. J. Bell, *J. Chem. Soc.*, 1874 (1935); L. Smith and J. Lindberg, *Ber.*, **64**, 505 (1931); J. W. Pette, *ibid.*, **64**, 1567 (1931); H. O. L. Fischer and C. Taube, *ibid.*, **60**, 485 (1927).

[122] E. Fischer and C. Rund, *Ber.*, **49**, 93 (1916); C. G. Anderson, W. Charlton and W. N. Haworth, *J. Chem. Soc.*, 1329 (1929).

[123] K. Freudenberg and A. Wolf, *Ber.*, **58**, 300 (1925), E. H. Goodyear and W. N. Haworth, *J. Chem. Soc.*, 3136 (1927)

When a ring change is not possible, as with the glycosides under nonacidic conditions, or when a ring change will not provide two free hydroxyls, as with the pentoses, the second acetone molecule may condense with hydroxyl groups which are not on contiguous carbon atoms. Thus, methyl α-D-mannopyranoside reacts with acetone (free of methyl alcohol) containing hydrogen chloride to produce methyl 2,3-4,6-diisopropylidene-D-mannopyranoside.[124]

The isopropylidene groups are easily removed by dilute acids. In most instances, one of the groups is much more readily removed than the other; by selection of the proper conditions, one group is hydrolyzed and the monoisopropylidene sugar obtained. For diisopropylidene-D-glucose, the group in the 5,6 position is hydrolyzed more than forty times as rapidly as that in the 1,2 position.[125] Hence treatment with acetic acid, or nitric acid and ethyl acetate, produces 1,2-isopropylideneglucose from the diisopropylidene derivative.[126] The remaining group is hydrolyzed more than 500 times as rapidly as the alkyl glycosides and disaccharides.[125]

The 1,2-isopropylidene sugars have a linkage formed from two acetal hydroxyls, as in sucrose. Hence, it would be expected that the group would be easily removed by acids. It is of interest that the 5,6-isopropylidene group is even more easily removed.

The structures of the isopropylidene sugars (acetone sugars) have been extensively investigated particularly by application of the methylation procedure.[127]

Diisopropylidene-D-glucose yields upon methylation and subsequent acid hydrolysis crystalline 3-methylglucose. The monoisopropylideneglucose obtained by the removal of one acetone group is substituted on the reducing carbon since it has no action on Fehling solution. Methylation produces monoisopropylidenetrimethylglucose which must have a furanose structure since, after acid hydrolysis and further methylation, methyl tetramethylglucofuranoside is formed. This evidence fixes the structure of the diisopropylideneglucose as having only the hydroxyl on carbon 3 free and as having a furanose ring; the acetone groups must then be in positions 1,2 and 5,6.[128] For the monoisopropylideneglucose the single acetone group is located at positions 1 and 2. A structural isomer, 1,2-3,5-diisopropylideneglucose,[129] results from the condensation of acetone with 6-substituted glucose.

Fructose condenses with acetone to form two structurally isomeric di-

[124] R. G Ault, W. N. Haworth and E. L. Hirst, J. Chem. Soc., 1012 (1935)
[125] K Freudenberg, W. Durr and H. v. Hochstetter, Ber., 61, 1735 (1928)
[126] H. W. Coles, L. D. Goodhue and R. M. Hixon, J. Am. Chem. Soc., 51, 523 (1929)
[127] W. N. Haworth in the "The Constitution of the Sugars;" Edward Arnold & Company, London (1929), presents an excellent account of this work
[128] C. G. Anderson, W. Charlton and W. N. Haworth J. Chem. Soc., 1329 (1929)
[129] H. Ohle and L. von Vargha, Ber., 62, 2425 (1929)

isopropylidenefructoses which have been designated as "alpha" and "beta" diacetonefructoses. This usage of alpha and beta has no relationship to the alpha and beta sugars and should be discarded when the structures of both have been determined unequivocally. The substance originally designated as the "alpha" isomer upon methylation yields a monomethyl derivative which on partial hydrolysis gives a monoisopropylidene-monomethyl-fructose and on complete hydrolysis a monomethylfructose. Since this mono-methylfructose is convertible to the same osazone as 3-methylglucose, the methyl group must be at position 3. The monoisopropylidene-3-methyl-fructose, after methylation and acid hydrolysis of the acetone and glycosidic methyl group, yields a trimethylfructopyranose, the ring structure being demonstrated by further methylation to tetramethylfructo*pyranose* (of known structure). This evidence places the free hydroxyl of the diisopro-pylidenefructose at position 3 and the oxygen ring between carbons 2 and 6. The "alpha" diacetone fructose is then 1,2–4,5-diisopropylidene-D-fructo-pyranose, and the "alpha" monoacetonefructose is 1,2-isopropylidene-fructopyranose.[127,130]

The second isomer, "beta" diacetonefructose (I), has neither hydroxyl 3 nor 6 free since the osazone of the monomethylfructose obtained after methylation and removal of the acetone groups is different from the osa-zones of the 3 and 6-methylglucoses. It is oxidized by alkaline permanganate to a monobasic acid (II) and, hence, one primary hydroxyl group must be free. This acid, after acid hydrolysis of the acetone residues and subsequent methylation, gives the same product (III) as that obtained by oxidation of methyl 1,3,4,5-tetramethylfructopyranoside (IV). Since the primary hy-

(I)   (II)

(IV)   (III)
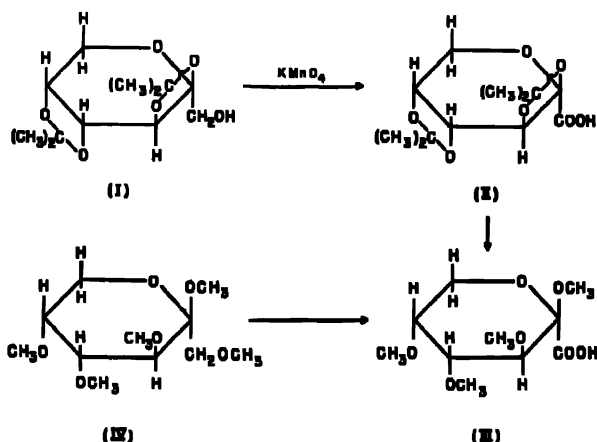
[130] K. Freudenberg and A. Doser, *Ber.*, **56**, 1243 (1923); C. G. Anderson, W. Charl-ton, W. N. Haworth and V. S. Nicholson, *J. Chem. Soc*, 1337 (1929).

droxyl of carbon 1 of the original substance must be unsubstituted and since the other carbons are involved in either ring formation or acetone bridges, the "beta" isomer probably is 2,3–4,5-diisopropylidenefructo-pyranose,[130, 131] but the 2,3–4,6-diisopropylidenefructofuranose structure is not eliminated.

Mono- and di-isopropylidene-glucoses when injected into animals are mainly eliminated in the urine, but an appreciable quantity of acetone is exhaled particularly in the case of the latter derivative.[132] The diisopropylidene derivative is toxic to rabbits and rats although the mono derivative is nontoxic.

E. Acetoacetic Ester Derivatives. Glucose condenses with acetoacetic ester, benzoylacetic ester and β-diketones in the presence of anhydrous zinc chloride to give derivatives of furan.[133] The constitution of some of the compounds has been demonstrated by oxidations with periodic acid and with lead tetraacetate. When heated in boiling solutions of dilute acids, a rearrangement takes place and a pyran ring is formed.

Glucose   Acetoacetic
             ester
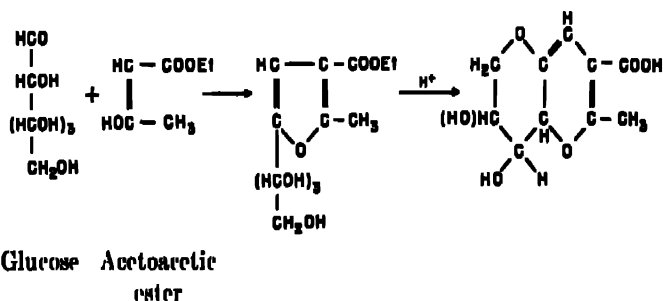
Compounds of this type have considerable biochemical interest because they may explain the antiketogenic action of glucose in preventing the formation of ketone bodies during animal metabolism. *In vitro* studies have shown that the oxidation of the ester of acetoacetic acid by hydrogen peroxide proceeds much more rapidly in the presence of glucose than in its absence.[134]

[131] H. Ohle, *Ber*, *58*, 2577 (1925)

[132] E. Dingemanse and E. Laqueur, *Enzymologia*, *4*, 57 (1937)

[133] E. S. West, *J. Biol. Chem*, *66*, 63 (1925); *74*, 561 (1927); *113*, 43 (1936); A. Muller and I. Varga, *Ber.*, *72*, 1993 (1939); G. Gonzáles, *Anales soc. españ física quím.*, *32*, 815 (1934); J. K. N. Jones, *J. Chem Soc*, 116 (1945)

[134] P. A. Shaffer, *J. Biol. Chem*. *47*, 433, 440 (1921)

CHAPTER VI

THE POLYOLS*

PART I

ACYCLIC POLYOLS (GLYKITOLS)

The designation "polyols" introduced here is synonymous with the longer, customary term, polyhydric alcohols. The polyols may conveniently be divided into two general classes, the acyclic linear polyols which which will be considered in Part I and the alicyclic or cyclitols which will compose Part II. Examples of each class are sorbitol and *meso*-inositol.

The first group, also called sugar alcohols or glykitols (see p. 17) is the larger from the standpoint of the number of known compounds although only a minority occur in nature.[1] The acyclic polyols may be subdivided on the basis of the number of hydroxyls in the molecule into tetritols, pentitols, hexitols, etc

As a group the acyclic polyols are crystalline bodies covering a wide range in melting point and in taste ranging from faintly sweet to very sweet The distribution in nature apparently is limited only to plants of the higher as well as lower orders The polyols found in the mannas and exudates of certain plants are sometimes of secondary origin as a result of the action of bacteria on carbohydrates in the exudate. Several anhydro hexitols (Chapter VIII) and glycosides in which hexitols supply the aglycon groups (Chapter XI) occur in plant products.

Polyols, particularly glycerol, ethylene glycol, sorbitol and mannitol have widespread commercial application, frequently as a result of their solubility in water and their hygroscopic properties. The organic mono esters, particularly of long-chain fatty acids, may have surface active properties which make the products of interest as emulsifiers and foam-producing agents, but the usual conditions of commercial esterification produce anhydro derivatives simultaneously (Chapters IV and VIII) Nitrate esters are important compounds as explosives The acetal derivatives (Chapter V) have been extensively prepared and studied, but as yet have found no practical application.

* Prepared by Dr. Sol Soltsberg, Atlas Powder Co., Wilmington, Del

[1] Although ethylene glycol and glycerol (diol and triol, respectively) may be properly classified as sugar alcohols they will not be considered here as they have been suitably covered in several books dealing exclusively with these compounds See: J W Lawrie, "Glycerol and the Glycols," A. C. S. Monograph 44, Chemical Catalog Co., New York (1928); G. Leffingwell and M Lesser. "Glycerin," Chemical Pub Co, New York (1945).

Mannitol, sorbitol and glycerol are nontoxic precursors of glycogen in the animal body, but in a general way, the anhydrides are not metabolizable.[2]

1. Configurations, Occurrence and Preparation

As indicated above, a convenient classification, which will be adhered to below, is that based on the number of hydroxyl groups in the molecule.

A. Tetritols. All of the theoretically possible tetritols are known. The configurations shown will be limited only to the D member of an enantiomorphic pair (For a discussion of configurational prefixes, see p. 18.)

$$
\begin{array}{cc}
\text{H}_2\text{COH} & \text{H}_2\text{COH} \\
| & | \\
\text{HOCH} & \text{HCOH} \\
| & | \\
\text{HCOH} & \text{HCOH} \\
| & | \\
\text{H}_2\text{COH} & \text{H}_2\text{COH} \\
\text{(I)} & \text{(II)} \\
\text{D Threitol} & \text{Erythritol} \\
\text{(l-Erythritol)} & \text{(meso-Erythritol)}
\end{array}
$$
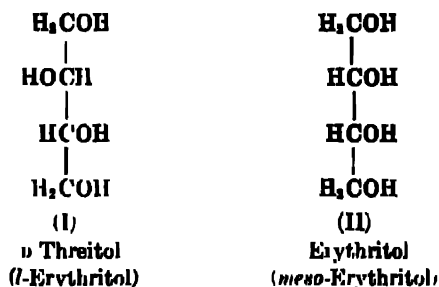
D-Threitol (I), m.p. 88°; $[\alpha]_D$ +4.3° (H$_2$O); dibenzylidene derivative, m.p. 231°. This alcohol is not found in nature. It was synthesized by Maquenne[3] from D-xylose by way of the Wohl degradation and sodium amalgam reduction.

L-Threitol, m.p. 88–89°; $[\alpha]_D$ −4.16°. Like its enantiomorph this compound is purely synthetic. It was obtained by Bertrand[4] from erythritol by bacterial oxidation to L-erythrulose followed by reduction with sodium amalgam.

D,L-Threitol has been characterized only as the dibenzylidene derivative, m.p. 217-9°. The racemate was synthesized by novel means starting from 3,4-epoxy-1-butene.[5] The steps required are given as follows:

$$
\begin{array}{ccc}
\begin{array}{c}
\text{H} \quad \text{H} \\
| \quad | \\
\text{H}_2\text{C} - - \text{C} \quad \text{C}\!\!=\!\!\text{CH}_2 \\
\diagdown \quad \diagup \\
\text{O}
\end{array}
& \xrightarrow{\text{H}_2\text{O}} &
\begin{array}{c}
\text{H} \quad \text{H} \\
| \quad | \\
\text{H}_2\text{C}\!-\!\text{C} - \text{C}\!\!=\!\!\text{CH}_2 \\
| \quad | \\
\text{O} \quad \text{O} \\
| \quad | \\
\text{H} \quad \text{H} \\
\text{Erythrol}
\end{array}
& \xrightarrow{\text{Ba(MnO}_4)_2}
\begin{array}{c}
\text{D,L-Threitol} \\
\text{and} \\
\text{Erythritol}
\end{array}
\end{array}
$$
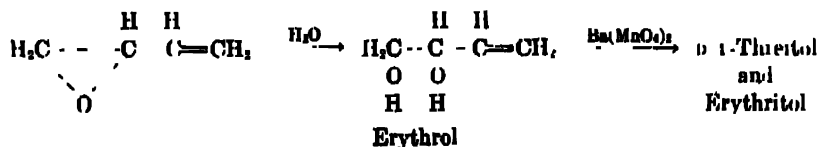
[2] See: C. J. Carr and J. C. Krantz, Jr., *Advances in Carbohydrate Chem*, *1*, 175 (1945).

[3] L. Maquenne, *Compt. rend., 130*, 1402 (1900).

[4] G. Bertrand, *Compt. rend., 130*, 1472 (1900).

[5] H. Pariselle, *Compt. rend., 150*, 1343 (1910)

Erythritol (II) m.p. 120°; dibenzylidene derivative, m.p. 201°. This tetritol occurs in nature in certain algae,[6] lichens[7] and grasses.[8]

In addition to the classical methods (reduction of appropriate aldose or ketose),[3,9] erythritol was obtained synthetically from 3,4-epoxy-1-butene[8] and from epichlorhydrin through the following series of steps:[10]

$$H_2C \underset{O}{\overset{H}{\diagdown\diagup}} C-CH_2Cl \xrightarrow{HCN} NC-CH_2-CHOH-CH_2Cl$$

Epichlorhydrin

$$\downarrow PCl_5$$

$$NC-CH_2-CHCl-CH_2Cl$$

$$\downarrow \text{aqueous } Na_2CO_3$$

$$\text{Erythritol} \xleftarrow{Na\ Hg} O=\underset{\underset{\text{HO}\quad\text{OH}}{|\quad\ \ |}}{\overset{H\ \ H}{C-C-C}}-CH_2 \xleftarrow{Ba(MnO_4)_2} O=\underset{\underset{-O-}{|\qquad\qquad}}{\overset{H\ \ H}{C-C=C}}-CH_2$$
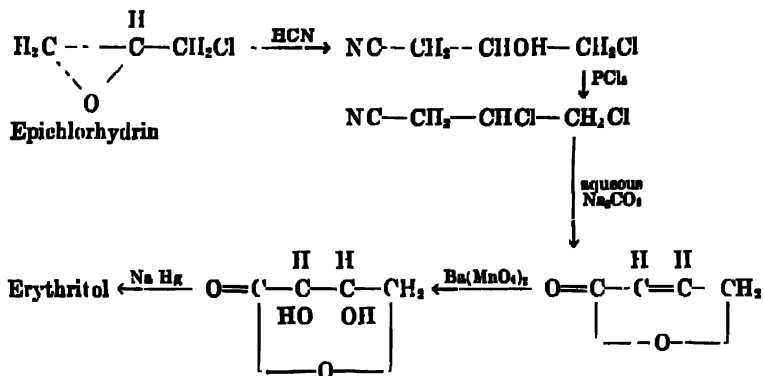
Griner[11] also describes a very interesting synthesis from butadiene. More recently Glattfeld and Stack[12] obtained erythritol by the high pressure reduction of butyl erythronate.

B. Pentitols. All of the pentitols predicted by theory are known. As in the case of the tetritols, only the D configurations of the optically active polyols will be indicated.

H₂COH	H₂COH	H₂COH
H_2COH	H_2COH	H_2COH
HOCH	HCOH	HCOH
HCOH	HOCH	HCOH
HCOH	HCOH	HCOH
CH_2OH	CH_2OH	CH_2OH
(III)	(IV)	(V)
D-Arabitol	Xylitol	Ribitol (adonitol)
(D-lyxitol)	(meso)	(meso)
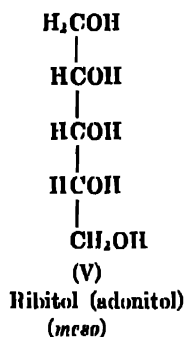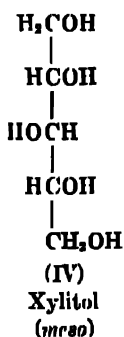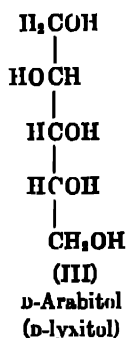
[6] M. Bamberger and A. Landsiedl, *Monatsh.*, **21**, 571 (1900); J. Tischer, *Z. physiol. Chem.*, **243**, 103 (1936).

[7] O. Hesse, *Ann.*, **117**, 297 (1861); *J. prakt. Chem.*, [2] **92**, 125 (1915); A. Goris and P. Ronceray, *Chem. Zentr.*, **78**, I, 111 (1907).

[8] A. W. Hofmann, *Ber.*, **7**, 508 (1874).

[9] O. Ruff, *Ber.*, **32**, 3677 (1899).

[10] R. Lespieau, *Bull. soc. chim.*, [4] **1**, 1112 (1907).

[11] G. Griner, *Bull. soc. chim.*, [3] **9**, 218 (1893).

[12] J. W. E. Glattfeld and A. M. Stack, *J. Am. Chem. Soc.*, **59**, 753 (1937).

D-Arabitol (III), m.p. 102°; $[\alpha]_D$ +7.82° (borax); pentaacetate, m.p. 76°. This polyol is rather rare in nature but has been found in certain lichens.[13] It is also found in the mushroom, *Fistulina hepatica*, to the extent of 9.5% on the dry weight.[14]

Synthetically, D-arabitol has been obtained by the reduction of either D-arabinose[15] or D-lyxose[16] by means of sodium amalgam.

L-Arabitol. The reported physical constants agree with those given for the D form except that the rotation in borax solution is somewhat smaller ($[\alpha]_D$ − 5.4°).[17] It does not occur naturally and has been prepared by the reduction of L-arabinose[18] and by employing the Cannizzaro reaction with L-arabinose in the presence of nickel.[19]

D,L-Arabitol, m.p. 105°; pentaacetate, m.p. 95°. The racemate is not found in nature and, of course, can be prepared from an equimolecular mixture of the enantiomorphs. It has been obtained synthetically along with ribitol by Lespieau.[20] The synthetic approach used by Lespieau is of general application and is equivalent to a total synthesis. The steps taken are as follows:

$$CH_2=CHCHO \xrightarrow{Cl_2} CH_2Cl-CHCl-CHO \xrightarrow{CH\equiv CMgBr}$$

$$\begin{array}{c} CH_2Cl \\ | \\ CHCl \\ | \\ CHOH \\ | \\ C \\ \| \\ CH \end{array} \Bigg\downarrow KOH$$

$$RCH=CH_2 \xleftarrow{H_2} CH_2OAc-(CHOAc)_3-C\equiv CH \xleftarrow[AgOAc]{AcO} CH_2Cl-CHCHC\equiv CH$$

$$\Bigg\downarrow \begin{array}{c} AgClO_3 \\ OsO_4 \end{array}$$

$$RCHOHCH_2OH \xrightarrow{AcO} D,L-Arabitol\ pentaacetate\ and\ ribitol\ pentaacetate$$
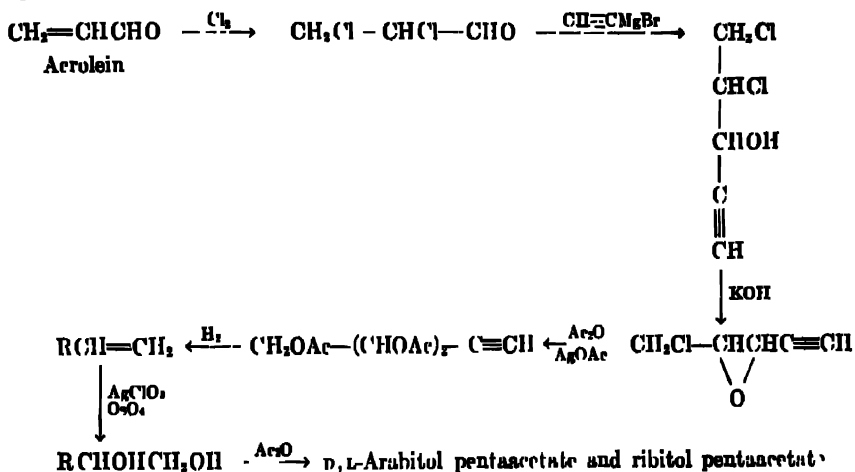
Xylitol (IV), m.p. 61–61.5° (metastable modification), 93–94.5° (stable modification); dibenzylidene derivative, m.p. 175°. Xylitol is not found in

[13] Y. Asahina and M. Yanagita, *Ber.*, **67**, 799 (1934); T. J. Nolan, J. Keane and V. E. Davidson, *Sci. Proc. Roy. Dublin Soc.*, **22**, 237 (1910).

[14] M. Frèrejacque, *Compt. rend.*, **208**, 1123 (1939).

[15] O. Ruff, *Ber.*, **32**, 550 (1899).

[16] O. Ruff and G. Ollendorf, *Ber.*, **33**, 1798 (1900).

[17] E. Fischer and R. Stahel, *Ber*, **24**, 538 (1891).

[18] H. Kiliani, *Ber.*, **20**, 1234 (1887).

[19] M. Delépine and A. Horeau, *Bull. soc. chim.*, [5] **4**, 1524 (1937).

[20] R. Lespieau, *Advances in Carbohydrate Chem.*, **2**, 107 (1946); *Compt. rend.*, **203**, 145 (1936).

nature despite the abundance of its parent aldose, D-xylose (wood sugar). Although xylitol has been known for about 50 years,[17, 21] it had never been obtained crystalline until Wolfrom and Kohn[22] obtained the metastable form in 1942. Shortly, thereafter, the stable modification was reported by Carson, Waisbrot and Jones[23] who were able to go from one form to the other at will. In the more recent work, D-xylose was reduced over nickel under pressure, whereas Fischer employed sodium amalgam. As pointed out by Hudson,[24] pressure hydrogenation in general will yield a purer product than that obtained by the sodium amalgam reduction of sugars.

Xylitol is one of the sweetest polyols known.

Ribitol (adonitol) (V), m.p. 102°; dibenzylidene derivative, m.p. 164 5°. Ribitol thus far has been found in nature in only two plants, *Adonis vernalis*[25] and *Buplturum falcatum* root (the Chinese drug, Chei-Hou).[26] In a combined form it is a constituent of riboflavin (vitamin B_2) (see also Chapter IX).

Synthetic ribitol has been prepared by the reduction of L-ribose with sodium amalgam.[27] Oddly enough, whereas L-ribose is a synthetic pentose, ribitol does not appear to have been prepared from the naturally occurring D-ribose. Lespieau[20] obtained ribitol along with D,L-arabitol by his novel synthesis, mentioned previously.

C. Hexitols. There are ten stereoisomeric hexitols possible and all are known. Again only the formulas of the D-enantiomorphs and *meso* compounds will be given.[29]

Sorbitol[19] (D-sorbitol, D-glucitol) (VI), m.p. 93 ' (labile form), 97.5' (stabile form); $[\alpha]_D$ --1.98° (H_2O), $[\alpha]_D$ +6.5° (borax); hexaacetate, m.p 101-2°.

Sorbitol is one of the most widespread of all the naturally occurring polyols. It is found exclusively in plants, apparently ranging from algae (seaweed) to the higher orders especially in the fruit and berries, but not in grapes or only to an insignificant extent. It was discovered in the fresh juice of the berries of the mountain ash (*Sorbus aucuparia* L.) by Boussingault in 1872; sorbose had been found earlier in the fermented juice by

[21] E. Fischer, *Ber.*, **27**, 2487 (1894)

[22] M. L. Wolfrom and E. J. Kohn, *J. Am. Chem. Soc*, **64**, 1739 (1942).

[23] J. F. Carson, S. W. Waisbrot and F. T. Jones, *J. Am. Chem. Soc.*, **65**, 1777 (1943)

[24] C. S. Hudson, *Advances in Carbohydrate Chem*, **1**, 21, (1945).

[25] W. V. Podwyssozki, *Arch Pharm.*, **227**, 141 (1889); E. Merck, *ibid.*, **231**, 129 (1893)

[26] F. Wessely and S. Wang, *Monatsh.*, **72**, 168 (1938).

[27] E. Fischer, *Ber.*, **26**, 633 (1893).

[20] R. Lespieau, *Bull. soc. chim.*, [5] **5**, 1638 (1938).

[29] For a more extensive discussion of the names and of the configurational prefixes, see Chapters I and II.

Pelouze (1852). In the red seaweed, *Bostrychia scorpoides*, sorbitol is found to the extent of 13.6%,[30] and in *Sorbus commixta* Nedlund, to the amount of 10%.[41] Strain[32] has examined a large number of plants and determined their sorbitol contents Fruit of the plant family Rosaceae, such as pears, apples, cherries, prunes, peaches and apricots, contain appreciable amounts of sorbitol [32]

$$
\begin{array}{cccc}
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
| & | & | & | \\
\text{HCOH} & \text{HOCH} & \text{HOCH} & \text{HOCH} \\
| & | & | & | \\
\text{HOCH} & \text{HOCH} & \text{HOCH} & \text{HCOH} \\
| & | & | & | \\
\text{HCOH} & \text{HCOH} & \text{HOCH} & \text{HOCH} \\
| & | & | & | \\
\text{HCOH} & \text{HCOH} & \text{HCOH} & \text{HCOH} \\
| & | & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
(VI) & (VII) & (VIII) & (IX) \\
\text{Sorbitol} & \text{D Mannitol} & \text{D-Talitol} & \text{D Iditol} \\
\text{(D Glucitol)} & & &
\end{array}
$$

$$
\begin{array}{cc}
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
| & | \\
\text{HCOH} & \text{HCOH} \\
| & | \\
\text{HOCH} & \text{HCOH} \\
| & | \\
\text{HOCH} & \text{HCOH} \\
| & | \\
\text{HCOH} & \text{HCOH} \\
| & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
(X) & (XI) \\
\text{Galactitol} & \text{Allitol} \\
(meso) & (meso)
\end{array}
$$
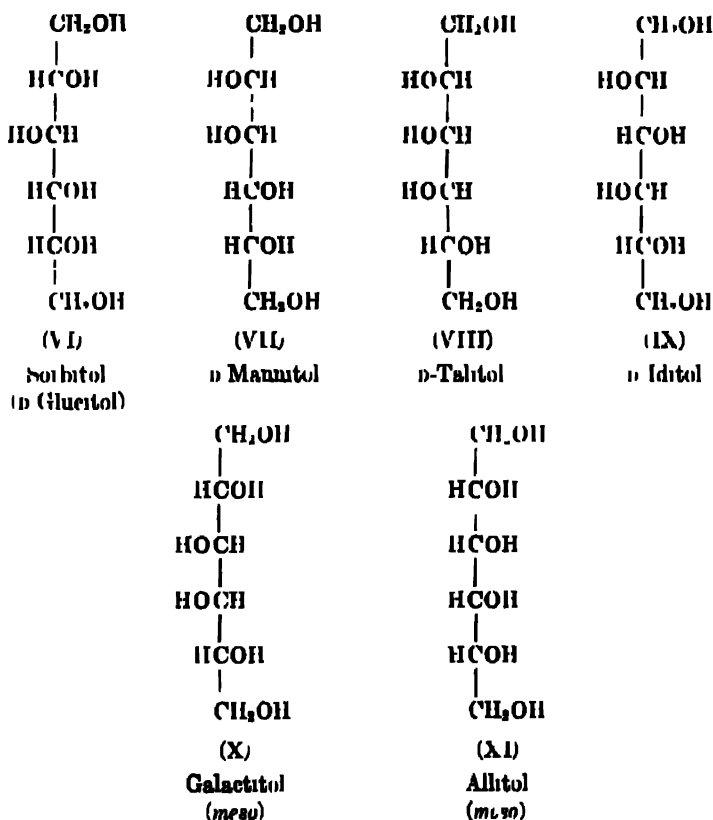
Sorbitol has been obtained synthetically from D-glucose by reduction with sodium amalgam and by pressure hydrogenation using platinum, Raney nickel or Adkins-type nickel catalyst It has also been obtained by the electrolytic reduction of glucose, by the Cannizzaro reduction of glucose in the presence of a hydrogenation catalyst and by the pressure hydrogenation of gluconic lactones.

Of the various processes, pressure hydrogenation and electrolytic reduc-

[30] P. Haas and T. G. Hill, *Biochem J* , *26*, 987 (1932)
[31] Y. Asahina and H. Shimoda, *J. Pharm. Soc Japan*, *50*, 1 (1930)
[32] H. H. Strain, *J Am. Chem. Soc.*, *59*, 2264 (1937); *56*, 1756 (1934)
[33] C Vincent and Delachanal, *Compt rend.*, *109*, 676 (1889)

tion are the industrially preferred operations.[14] As a result of these two processes and the advent of cheap crystalline glucose of high purity, sorbitol is no longer a chemical curiosity but an established cheap article of commerce generally sold in aqueous solution.

Thus far, aside from patents, very little has been published concerning the commercial pressure hydrogenation process. It is believed to be limited mostly to a batch type process although a continuous method should also prove feasible since a continuous process has been developed in Germany for the reduction of glucose to "glycerogen," a mixture mainly of ethylene and propylene glycols but containing some glycerol and higher polyols.

In one commercial batch process, an Adkins-type catalyst is used (a nickel salt is precipitated on a porous clay support, dried, ground and reduced in a furnace). The catalyst is suspended in an aqueous glucose solution, and the reduction is carried out in an autoclave at about 150°C. under 100 to 150 atmospheres pressure.

In the electrolytic process, which is continuous, a glucose solution containing sodium sulfate as the electrolyte is reduced at an amalgamated lead or zinc cathode. The anolyte is sulfuric acid contained in alundum diaphragms. The present electrolytic process is a refinement of the original Creighton process. [34b,35]

D-Gulitol (L-sorbitol, L-glucitol), m.p. 89-91°; $[\alpha]_D^{20}$ +2°; hexaacetate, m.p. 98-99°; $[\alpha]_D^{27}$ −10° (CHCl$_3$). This polyol is not found in nature. It has been synthesized by the reduction of D-gulose by catalytic high-pressure hydrogenation[36] and from D-sorbose by means of sodium amalgam.[37]

D,L-Gulitol (D,L-glucitol, D,L-sorbitol), m.p. 135-7°; hexaacetate, m.p. 117-18°. The racemic polyol has been made by mixing equimolecular quantities of the two components and has also been isolated in small yield from a commercial sorbitol prepared by the electrolytic reduction of D-glucose under alkaline conditions.[36]

D-Mannitol (VII), m.p. 166°; $[\alpha]_D^{25}$ −0.21° (H$_2$O); $[\alpha]_D$ +28.3° (borax);

[34] There are a number of patents based on these two processes:
  a. Pressure hydrogenation:
    I. G. Farbenind. A.-G., British Patent 354,196, May 1, 1930; French Patent 694,424, April 25, 1930; J Mueller and U. Hoffman, U. S Patent 1,990,245, Feb. 5, 1935; A. W. Lachar, U. S. Patent 1,963,999, June 26, 1934; R. S. Rose, Jr., U. S. Patent 2,292,293, Aug. 4, 1944.
  b. Electrolytic hydrogenation:
    H. J. Creighton, U. S. Patents 1,712,051, 1,712,952, May 14, 1929; G. A. Krikhgof and O. I. Korzina, Russ. Patent 51,750, Sept. 20, 1937; R. A. Hales, U. S. Patent 2,289,189, 2,289,190, July 7, 1942; 2,300,218, Oct. 27, 1942.
[35] R. L. Taylor, *Chem. & Met. Eng.*, *44*, 588 (1937); H. J. Creighton, *Can. Chem. Process Inds.*, *26*, 690 (1942); *Trans. Electrochem. Soc.*, *75*, 389 (1939).
[36] M. L. Wolfrom, B. W. Lew, R. A. Hales and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, *68*, 2342 (1946).
[37] C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, *19*, 7 (1900).

hexaacetate, m.p. 125°. Like sorbitol, D-mannitol is widespread among plants. However, unlike sorbitol, it is frequently found in exudates of plants. It is probably for this reason and because D-mannitol is a highly crystalline and only moderately soluble polyol that it was the first crystalline polyol discovered.[38] It was isolated from the manna of the flowering or manna ash, *Fraxinus ornus*. It is also found in the exudates of the olive and plane trees, constituting 80 to 90% of the latter's exudate.[39] For a time D-mannitol was obtained commercially in Sicily from the sap of *Fraxinus rotundifolis*.

D-Mannitol has been synthesized by several methods. At present the most commercially important method is the electroreduction of D-glucose under more or less alkaline conditions; D-sorbitol is formed simultaneously. Depending on the alkalinity, over 20% of the glucose can be converted to D-mannitol in this manner.

For the best laboratory preparation D-mannitol is obtained by the catalytic reduction of D-mannose obtained from the vegetable ivory nut (see Chapter III), or by the reduction of D-fructose or invert sugar.

Among other syntheses are the catalytic reduction of D-mannonic δ-lactone[40] and the microbiological conversion of D-glucose or sucrose. A species of *Aspergillus* is capable of producing a 50% yield of D-mannitol from D-glucose,[41] whereas based on fructose content, *B. coli*, *B. freundi* and *B. paratyphii* are reported to give 92 to 93% conversion of sugar-beet diffusion juice, 91% of carob beans and 92.7% of grape juice.[42]

L-Mannitol does not occur in nature. It has been obtained by the reduction of L-mannose with sodium amalgam[43] or the catalytic reduction of L-mannonic lactone with the aid of a platinum catalyst containing a little iron and under a pressure of 80 atmospheres.[44]

D,L-Mannitol (α-acritol), m.p. 168°; tribenzylidene derivative, m.p. 190-2°. It has been obtained by the reduction of α-acrose (see p. 112). Divinylglycol from acrolein was the starting point of Lespieau and Wiemann.[20][45] The glycol was obtained from acrolein by reduction with the zinc-copper couple and was then oxidized with silver chlorate and osmium tetroxide to D,L-mannitol. Allitol was obtained simultaneously. It is apparent, therefore, that this divinylglycol must be a mixture of diols in which the hydroxyls are *cis* and *trans*.

[38] Proust, *Ann. chim. phys.*, [1] 57, 144 (1806).
[39] E. Jandrier, *Compt. rend.*, 117, 498 (1893).
[40] J. W. E. Glattfeld and G. W. Schimpff, *J. Am. Chem. Soc.*, 57, 2204 (1935).
[41] J. H. Birkinshaw, J. H. V. Charles, A. Hetherington and H. Raistrick, *Trans. Roy. Soc. (London)*, B220, 153 (1931).
[42] V. Boleato and G. Pasquini, *Ind. saccar. ital.*, 32, 408 (1939).
[43] E. Fischer, *Ber.*, 23, 375 (1890).
[44] E. Baer and H. O. L. Fischer, *J. Am. Chem. Soc.*, 61, 761 (1939).
[45] R. Lespieau and J. Wiemann, *Compt. rend.*, 194, 1946 (1932); *Bull. soc. chim.*, [4] 53, 1107 (1933).

Another interesting synthetic approach which also constitutes a total synthesis was accomplished by Pace.[46] Sodium acetoacetic ester was oxidized with iodine, the product saponified and carbon dioxide eliminated to give 2,5-hexanedione. The diketone was reduced to the diol, which was transformed to the dibromide. The hexadiene was formed and converted to the tetrabromide and subsequently to the hexabromide, which upon treatment with alcoholic potassium hydroxide gave D,L-mannitol.

D-Talitol (VIII), m.p. 86°; $[\alpha]_D^{15}$ +3.05° (H_2O); tribenzylidene derivative, m.p 205 6°. D-Talitol does not occur in nature. It was obtained crystalline by Bertrand and Bruneau[47] who repeated E. Fischer's earlier synthesis (reduction of D-talonic lactone with sodium amalgam).

L-Talitol, m.p. 87 88 , $[\alpha]_D^{20}$ −2.29° (H_2O) was the last of the hexitols to be synthesized. It was obtained by the reduction of L-altrose with a supported nickel catalyst at 2000 lbs. pressure and 100 °C.[48]

D,L-Talitol, m.p. 95–6 , does not occur in nature. It has been synthesized by mixing the enantiomorphs in equimolecular amounts.[49] As pointed out by these investigators, the melting point does not agree with that (66 67°) reported by E. Fischer[19] who oxidized galactitol with lead peroxide and reduced the product to the polyol which was converted to the tribenzylidene derivative, m.p. 205 6 ; the hexitol was recovered from the latter deriva tive. It was suggested that Fischer's product was either impure or a lower melting polymorph. The preparation of the tribenzylidene derivative of the synthetic mixture would provide a second comparison of the two products.

D-Iditol (IX), m.p. 73.5 ; $[\alpha]_D^{20}$ +3.5′ (H_2O); hexaacetate, m.p. 121.5 , $[\alpha]_D^{20}$ +25.3 ($CHCl_3$). This polyol does not occur in nature It has been synthesized by the reduction of D-idose[50] and D-sorbose,[37] the latter also producing L-sorbitol.

L-Iditol (sorbierite), m.p. 73.5°; $[\alpha]_D$ −3.53′ (H_2O); hexaacetate, m.p. 121.5 , $[\alpha]_D^{15}$ 25.65° ($CHCl_3$); tribenzylidene derivative, m.p. 242°. This polyol appears to be the rarest of the naturally occurring hexitols. It has been isolated only from the mother liquor after removing D-sorbitol by fermenting the juice of the mountain ash berry (*Sorbus aucuparia*). It was at first thought to be an octitol but Bertrand definitely established it as a hexitol.[51] It has been synthesized by the reduction of L-sorbose to D-sorbitol

[46] E. Pace, *Arch. farmacol. sper.*, *45*, 167 (1926)

[17] G. Bertrand and P. Bruneau, *Compt. rend.*, *146*, 482 (1908); *Bull. soc. chim* , [4] *3*, 495 (1908).

[48] F. L. Humoller, M. L. Wolfrom, B. W. Lew and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, *67*, 1226 (1945).

[49] E. Fischer, *Ber.*, *27*, 1528 (1894).

[50] G. Bertrand and A. Lanzenberg, *Compt. rend.*, *143*, 291 (1906); E. Fischer and W. Fay, *Ber.*, *28*, 1975 (1895).

[51] G. Bertrand, *Bull. soc. chim.*, [3] *33*, 166, 264 (1905).

and L-iditol; the sorbitol was removed by fermentation with sorbose bacteria and the nonfermentable L-iditol was isolated as the tribenzylidene compound.

Although reduction of L-sorbose appears to yield equimolecular amounts of the two hexitols, reduction of pentaacetyl-keto-L-sorbose appears to favor the formation of L-iditol. A 60% yield of L-iditol hexaacetate was obtained when pentaacetyl-keto-L-sorbose was hydrogenated over platinum catalyst in absolute ether at four atmospheres pressure. The hydrogenated product was further acetylated to the hexaacetate and fractionally crystallized.[52] A 90% yield is claimed when the reduction is carried out in alcohol using Raney nickel and atmospheric pressure at room temperature.[53]

Galactitol (dulcitol) (X), m.p. 188.5–189°; hexaacetate, m.p. 171°; like D-mannitol, has a widespread distribution and is found in plants ranging from red seaweed and pentose-fermenting yeast (*Torula utilis*) to the mannas of higher plant life. Madagascar manna appears to be relatively pure dulcitol.[54] At one time dulcitol was called melampyrum or melampyrite after *Melampyrum nemorosum* from which source it was first isolated.[55] It was found to the extent of about 2% in the fairly common American shrub, the burning bush[56] (*Euonymus atropurpureus*, Jacquin).

Galactitol has been synthesized from D-galactose by direct reduction and by the Cannizzaro process of Delépine and Horeau.[19] The equivalent of a total synthesis was achieved in the following manner:[57]

$$2 \ CH_2ClCHO \xrightarrow{\text{BrMgC} \equiv \text{CMgBr}} CH_2Cl \ CHOH - C \equiv C - CHOH - CH_2Cl$$

Chloroacetaldehyde

$$CH_2,OAc - CHOAc - CHOH - CHOH - CHOAc - CH_2OAc$$

$$\xrightarrow{\text{acetylate}} \text{Galactitol hexaacetate}$$
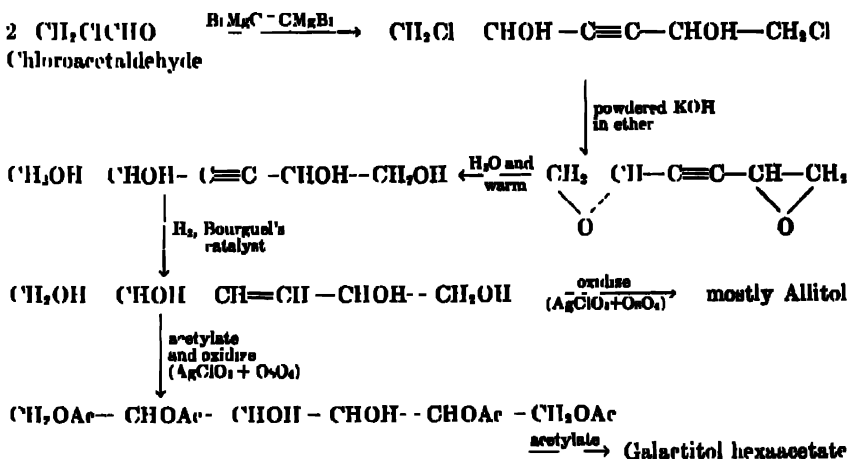
[52] F. B. Cramer and E. Pacsu, *J. Am. Chem. Soc.*, **59**, 1467 (1937).

[53] Y. Khouvine and G. Arragon, *Bull. soc. chim.*, [5] **5**, 1404 (1938).

[54] G. Bouchardat, *Ann. chim. phys.*, [4] **27**, 68 (1872).

[55] Hünefeld, *Ann.*, **24**, 241 (1837).

[56] See: H. Rogerson, *J. Chem. Soc.*, **101**, 1040 (1912).

[57] R. Lespieau and J. Wiemann, *Compt. rend.*, **198**, 133 (1934); R. Lespieau, *Bull. soc. chim.*, [5] **1**, 1374 (1934).

The diolization of the double bond in this series of reactions appears to be analogous to the diolization of the double bond of conduritol, a cyclo-hexenetetrol (see Cyclitol section).

When the hexenetetrol above was oxidized directly, the hydroxyls entered *cis* to the hydroxyls already present and allitol was the chief product; similarly alloinositol was obtained on oxidation of isopropylidene-conduritol diacetate with potassium permanganate. On the other hand, for the two fully acetylated tetrols (acyclic and cyclic), the hydroxyls entered *trans* to those already present giving galactitol and mucoinositol tetraacetates, respectively (p. 275).

Allitol (allodulcitol) (XI), m.p. 150–151°; hexaacetate, m.p. 61°; dibenzylidene derivative, m.p. 219 50°. Allitol does not occur in nature. It has been obtained along with D,L-mannitol by the oxidation of Griner's divinylglycol.[45] Wiemann[45] modified this synthesis by brominating Griner's divinylglycol instead of oxidizing it. He isolated a tetrabromide in which the hydroxyls were *cis*. Then, on debromination, the divinylglycol with exclusively *cis* hydroxyls was obtained which on oxidation with the silver chlorate, osmic acid reagent gave allitol with but a trace of galactitol.

An unequivocal synthesis of allitol was the reduction of D-allose with hydrogen and nickel catalyst.[59] The resulting hexitol agreed in its constants with the product made by Lespieau and Wiemann.

D. Heptitols.[60] Only ten of the sixteen theoretically possible heptitols are described in the literature. Of the ten, only two are found in nature and another may be identical with one already known. The configurations of

CH₂OH	CH₂OH	CH₂OH

$$
\begin{array}{ccc}
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
| & | & | \\
\text{HCOH} & \text{HOCH} & \text{HCOH} \\
| & | & | \\
\text{HCOH} & \text{HCOH} & \text{HOCH} \\
| & | & | \\
\text{HOCH} & \text{HOCH} & \text{HOCH} \\
| & | & | \\
\text{HCOH} & \text{HCOH} & \text{HCOH} \\
| & | & | \\
\text{HCOH} & \text{HCOH} & \text{HCOH} \\
| & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
\text{(XII)} & \text{(XIII)} & \text{(XIV)}
\end{array}
$$

(XII)	(XIII)	(XIV)
Gluco *gulo* heptitol (*meso*)	D-Gluco-D-*ido* heptitol	D Manno-D-*gala*-heptitol (D-Perseitol)
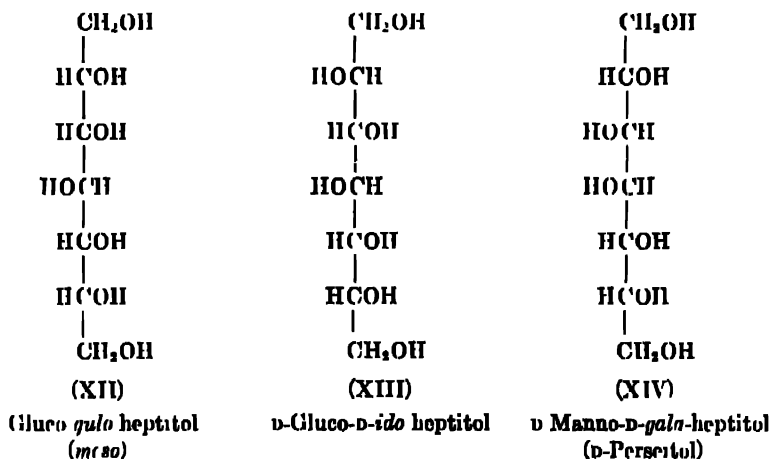
[45] J Wiemann, *Ann. chim.*, [11] 5, 267 (1936).

[59] M Steiger and T. Reichstein, *Helv Chim Acta*, 19, 184 (1936).

[60] For a review of the occurrence of these materials and their configuration, see:
C S Hudson, *Advances in Carbohydrate Chem*, 1, 1 (1945)

CH_2OH CH_2OH CH_2OH

HOCH   HOCH   HCOH

HOCH   HCOH   HCOH

HOCH   HOCH   HCOH

HCOH   HOCH   HOCH

HCOH   HCOH   HCOH

CH_2OH CH_2OH CH_2OH

(XV)   (XVI)   (XVII)

D-Manno D-*talo*-heptitol   D-Gala-L *gluco* heptitol   D Gulo-L-*talo*-heptitol
(D-Volemitol)
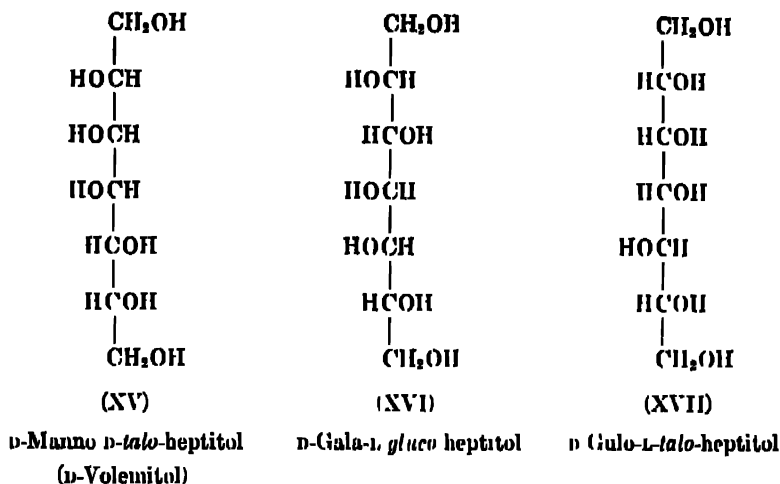
the D-series of the known optically active heptitols and the *meso* forms are illustrated.

The relationships between the naturally occurring heptoses and the corresponding heptitols and hexoses are shown below *:

```
                        D-Mannoheptulose
                      ↙        ↑        ↘
        D-Volemitol  ←  D-Mannose  →  D-Perseitol
        ↗          ↖              ↗            ↖
  D-Altrose   D-Sedoheptulose   L-Perseulose   L-Galactose
        ↘          ↙              ↘            ↙
  L Gulo D-talo heptitol  ←  L-Gulose  →  L-Gulo D gala-heptitol
```
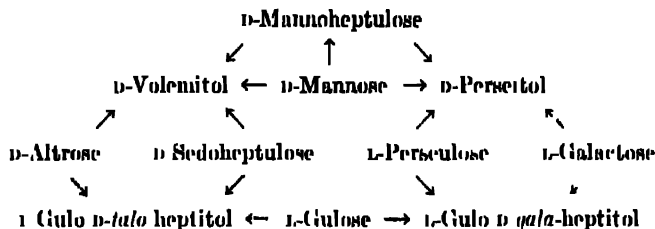
Gluco-*gulo*-heptitol (α-glucoheptitol) (XII), m.p. 129°; heptaacetate, m.p. 118°. This polyol does not occur naturally. It has been prepared by the reduction of α-glucoheptose obtained from D-glucose through the cyanohydrin synthesis.[61]

D-Gluco-D-*ido*-heptitol (D-β-glucoheptitol) (XIII), m.p. 129'; $[\alpha]_D^{20}$ +0.7° (H₂O); heptabenzoate, m.p. 181°; $[\alpha]_D^{20}$ +25.3° (CHCl₃). This is not a naturally occurring polyol; it has been synthesized by the reduction with sodium amalgam of D-β-glucoheptose as above.[61] L-Gluco-L-*ido*-hepti-

* The full arrows represent demonstrated conversions. For galactose and gulose, the arrows with dotted lines represent conversions carried out with the enantio morphic modifications. The conversion of D-altrose to the guloheptitol remains to be accomplished.

[61] E. Fischer, *Ann.*, **270**, 64 (1892); L. H. Philippe, *Ann chim. phys.*, [8] **26**, 289 (1912).

tol was obtained by hydrogenation of L-glucoheptulose in the presence of Raney nickel.[62]

D-Manno-D-*gala*-heptitol (D-perseitol, α-mannoheptitol) (XIV), m.p. 187° $[\alpha]_D^{20}$ −1.1° (H_2O); heptaacetate, m.p. 119.5°, $[\alpha]_D^{20}$ −13.3° ($CHCl_3$). This is one of the two naturally occurring polyols. It has been isolated from *Laurus persea* L. (avocado).[63] Its synthesis has been accomplished by the reduction of D-mannoheptose (D-manno-D-*gala*-heptose)[64] and of L-perseulose,[65] a naturally occurring ketoheptose.

L-Manno-L-*gala*-heptitol (L-perseitol, D-α-galaheptitol, D-gala-L-*manno*-heptitol); m.p. 187°; $[\alpha]_D$ +1.1° (H_2O); heptaacetate, m.p. 119°; $[\alpha]_D$ +13.4° ($CHCl_3$) This polyol is the enantiomorph of XIV and has been synthesized by the reduction of D-α-galaheptose It has also been obtained by the reduction of L-α-mannoheptose.[66]

D,L-Manno-*gala*-heptitol (D,L-perseitol), m.p. 205°. This is purely a synthetic mixture.

D-Manno D-*talo*-heptitol (D β mannoheptitol, α sedoheptitol, D volemitol) (XV), m.p., 153°; $[\alpha]_D^{20}$ +2.1°; heptaacetate, m.p 63°; $[\alpha]_D^{20}$ +36.1° ($CHCl_3$). This is the second of the naturally occurring heptitols and has been found in a mushroom, *Lactarius volemus*,[67] as well as in the roots of a number of varieties of plants of the primrose family.[68]

D-Volemitol has been synthesized by the reduction of D-β-mannoheptose (D-manno-D-*talo*-heptose)[64] and of the natural sedoheptulose.[69]

D-Gala-L-*gluco*-heptitol (D-β-galaheptitol, D-α-guloheptitol) (XVI), m.p 141.5°, $[\alpha]_D^{20}$ +2.1° (H_2O); heptaacetate, m.p. 118°; $[\alpha]_D^{20}$ +11.4° ($CHCl_3$). This synthetic polyol was obtained by the reduction of D-β-galaheptose[61] or D-α-guloheptose.[70]

L-Gala-D-*gluco*-heptitol, the enantiomorph of XVI, m.p. 141°, $[\alpha]_D^{20}$ −2.4° (H_2O); heptaacetate, m.p. 118 ; $[\alpha]_D^{20}$ − 11.4° ($CHCl_3$). This alcohol has been synthesized by the reduction of L-perseulose,[71] D-perseitol also being found.

D,L-Gala-*gluco*-heptitol, m.p. 136°; heptaacetate, m.p. 127°. This racemate was obtained by mixing equimolar amounts of the two components.

[62] W. D. Maclay, R. M. Hann and C S. Hudson, *J. Am Chem. Soc*, **64**, 1606 (1942)

[63] L. Maquenne, *Compt rend.*, **107**, 583 (1888), *Ann chim phys.*, [6] **19**, 5 (1890)

[64] G. Peirce, *J. Biol Chem.*, **23**, 327 (1915).

[65] G. Bertrand, *Compt rend.*, **149**, 225 (1909).

[66] W. S. Smith, *Ann.*, **272**, 182 (1893).

[67] E. Bourquelot, *J. pharm. chim.*, [6] **2**, 385 (1895).

[68] J. Bougault and G. Allard, *Compt. rend.*, **135**, 796 (1902)

[69] F. B LaForge, *J Biol. Chem.*, **42**, 375 (1920)

[70] F. B. La Forge, *J Biol. Chem.*, **41**, 251 (1920)

[71] R. M. Hann and C. S. Hudson, *J. Am. Chem Soc.*, **61**, 336 (1939); G. Bertrand *Compt. rend.*, **149**, 225 (1909).

D-Gulo-L-*talo*-heptitol (XVII), m.p. 128–129°; $[\alpha]_D^{20}$ +0.95° (H_2O), - 4.6° (sat. borax). This polyol was first synthesized by La Forge by the reduction of D-β-guloheptose and hence was called D-β-guloheptitol. He considered this substance to be optically inactive. However, it has since been shown to have a slight but definite optical activity [72]

L-Gulo-D-*talo*-heptitol is the enantiomorph of XVII. It was obtained conjointly with D-manno-D-*talo*-heptitol (D-volemitol) by Ettel [73] from the reduction of sedoheptulose. Hence, it must be the epimer of D-volemitol.

"α-Glucoheptulitol," m.p. 144°; $[\alpha]_D^{20}$ -2.24° (H_2O), heptaacetate, m. p. 116 7°. The configuration of this heptitol is still in doubt although it was obtained along with α-glucoheptitol (XII) by the sodium amalgam reduc tion of L-glucoheptulose [71] (For further discussion, see below.)

E. Octitols, Nonitols and Decitols. Four octitols, one nonitol and one decitol have been described. Of these only the configurations of the octitols are completely known.

The configurations, as far as are known, are represented in formulas XVIII to XXIII.

D-Gluco-L-*gala*-octitol (XVIII), m.p. 153°, $[\alpha]_D$ +2 4° (H_2O); octaace-tate, m.p. 88–89°, $[\alpha]_D$ +20.7° ($CHCl_4$). This polyol is not naturally occur-ring, and it has been synthesized by the reduction of the corresponding octoses obtained from D-glucose and from D-galactose by way of the cyano-hydrin synthesis [76]
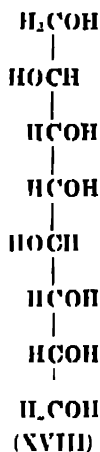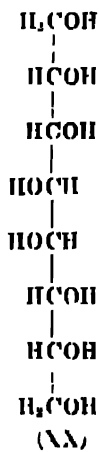
H_2COH	H_2COH	H_2COH
HOCH	HCOH	HCOH
HCOH	HCOH	HCOH
HCOH	HCOH	HOCH
HOCH	HOCH	HOCH
HCOH	HCOH	HCOH
HCOH	HCOH	HCOH
H_2COH	H_2COH	H_2COH
(XVIII)	(XIX)	(XX)
D Gluco L-*gala* octitol	D-Gluco L *talo* octitol	Manno-*manno*-octitol

[72] A T Merrill, W T Haskins, R M Hann and C S Hudson, *J. Am. Chem. Soc.,* **69**, 70 (1947)

[73] L Ettel, *Collection Czechoslov Chem. Commun.,* **4**, 513 (1932)

[74] G. Bertrand and G Nitzberg, *Compt rend ,* **186**, 1172, 1773 (1928); Y Khouvine and G Nitzberg, *ibid ,* **196**, 218 (1933).

[76] R. M Hann, A T Merrill and C. S. Hudson, *J. Am. Chem Soc.,* **66**, 1912 (1944).

```
   H₂COH              H₂COH              H₂COH
    |                  |                  |
   HOCH               HOCH (?)           HCOH (?)
    |                  |                  |
   HCOH               HOCH               HOCH   (?)
    |                  |                  |
   HCOH               HCOH               HOCH
    |                  |                  |
   HOCH               HCOH               HCOH
    |                  |                  |
   HOCH               HOCH               HCOH
    |                  |                  |
   HCOH               HCOH               HOCH
    |                  |                  |
   H₂COH              HCOH               HCOH
                       |                  |
   (XXI)              H₂COH              HCOH
                                          |
                      (XXII)             H₂COH

                                         (XXIII)
```

D-Gala-L-gala-octitol   α,α,α D-Gluconotol   α,α,α,α D Glucodecitol
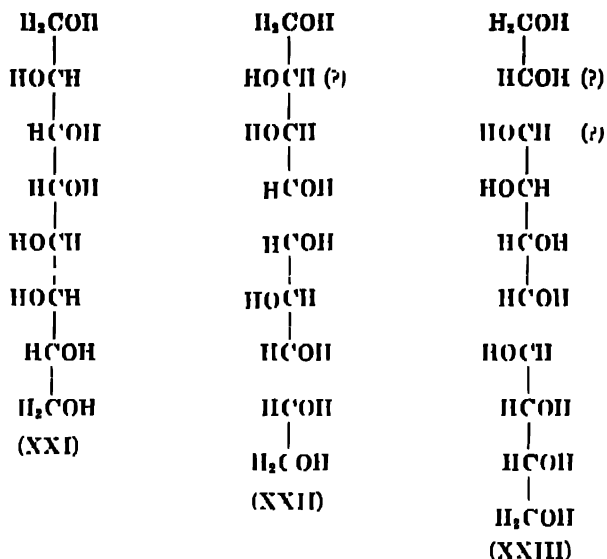
D-Gluco-L-talo-octitol (XIX), m p. 161 2°, $[\alpha]_D^{20}$ −0.8° (H₂O); octaacetate, m.p. 101-2°, $[\alpha]_D^{20}$ +17.1° (CHCl₃) This polyol was obtained by the reduction of the corresponding octose.

Manno-manno-octitol (XX), optically inactive, m.p. 262°; octaacetate, m.p. 166°. This was likewise obtained by reduction of the corresponding octose obtained from D-mannose by use of the cyanohydrin synthesis.[76]

D-Gala-L-gala-octitol (XXI), m.p. 230′, $[\alpha]_D$ 0.0° (H₂O), - 0.5 (borax); octaacetate, m.p. 141°, $|\alpha|_D$ +40.4° (CHCl₃). This synthetic polyol was obtained by reduction of the corresponding octose obtained from D-galactose by way of the cyanohydrin synthesis.[77]

α,α,α-D-Gluconotol (XXII), m.p. 198°, $[\alpha]_D^{15}$ +1.5° (H₂O). As the designation "α" indicates, this nonitol was obtained[61] by reduction of the most accessible nonose from D-glucose by way of the cyanohydrin synthesis. The configuration of this compound is not entirely known.

α,α,α,α-D-Glucodecitol (XXIII), m.p. 222°, $[\alpha]_D^{20}$ +1.2° (H₂O); decaacetate, m.p. 149 50°, $[\alpha]_D$ +16′ (CHCl₃). This product was synthesized by Philippe[61] in his extension of the cyanohydrin synthesis. Inasmuch as the configuration of the nonose preceding the decose is not completely known, the configurations of carbon atoms 2 and 3 of the decitol are not established.

[76] R. M. Hann, W. D Maclay, A. E. Knauf and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 1268 (1939)

[77] W. D Maclay, R M. Hann and C S. Hudson, *J. Am. Chem. Soc.*, **60**, 1035 (1938).

2. Proofs of Structure and Configuration

In the main, the proof of structure and of configuration of the stereo-isomeric polyols has been dependent on the proof of structure and of configuration of the parent sugar, determined as described in Chapter II. There have been occasions, however, when the configuration of the parent sugar and of the polyol derivable therefrom were simultaneously established by conversion of the sugar to the polyol. This has been true when the configuration of the carbohydrate has been known in part and the polyol resulting from the reduction of the carbohydrate was found to be optically inactive (*meso* structure). This type of proof has been of particular usefulness in the determination of the structures of the aldoses obtained by way of the cyanohydrin synthesis. Inasmuch as this synthesis invariably produces derivatives epimeric at carbon 2, the configuration of the 2-epimer likewise becomes known.

E. Fischer[78] used this type of proof in the establishment of the structures

CHO
|
HCOH
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH

cyanohydrin
synthesis

→ CHO
|
HCOH
|
HCOH
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH

NaHg₂

CH₂OH
|
HCOH
|
HCOH
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH
(XXIV)

→ CHO
|
HOCH
|
HCOH
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH

→ CH₂OH
|
HOCH
|
HCOH
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH
(XXV)

---
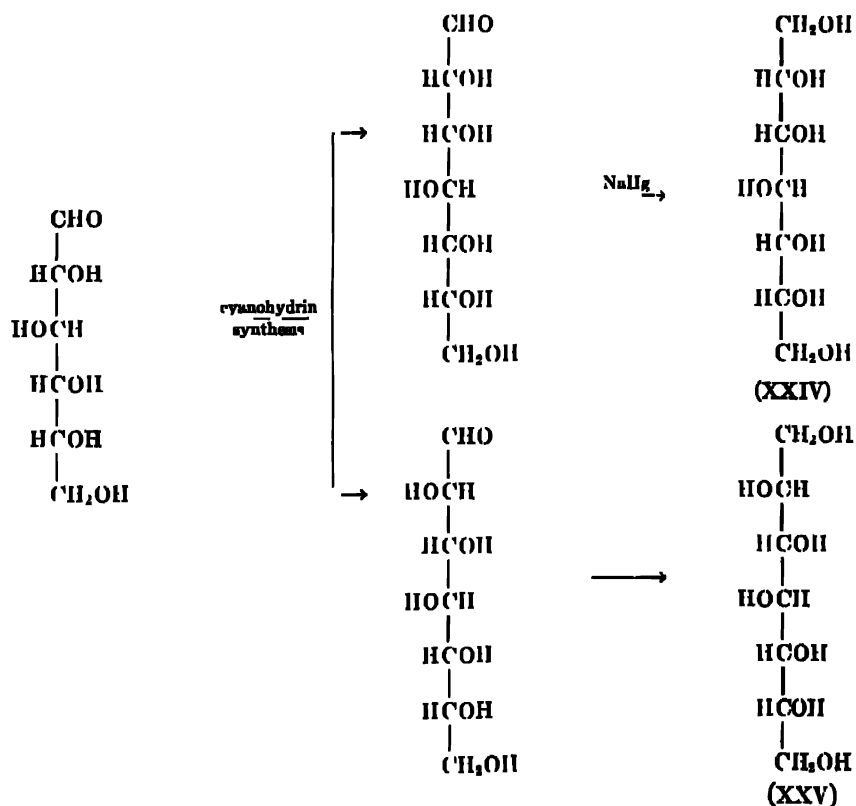
78 E. Fischer, *Ann*., *270*, 64 (1892) In this case, the proof was actually based on the corresponding saccharic acids.

of the heptoses and heptitols derived from D-glucose. Thus, D-g'ucose on application of the cyanohydrin synthesis yielded two heptoses, α-glucoheptose and β-glucoheptose. The glykitol (α-glucoheptitol) obtained by the reduction of α-glucoheptose was found to be optically inactive and hence must have a *meso* configuration. Of the two possible formulas (XXIV and XXV) only XXIV has a *meso* configuration, and it must represent α-glucoheptitol. Its systematic name is gluco-*gulo*-heptitol. The alcohol produced from the second product of the cyanohydrin synthesis can differ only in the configuration at carbon 2, and must be D-gluco-D-*ido*-heptitol (XXV). Hence, the configurations of the two heptitols and the two heptoses are established by this process.

Although the alcohols are superior to the saccharic acids as derivatives of aldoses for this type of structural proof because of their ease of crystallization, they have one marked deficiency, the low optical rotations. Hence it is possible to assign erroneously a *meso* configuration to a substance that is actually optically active.[79] Thus, β-sedoheptitol (D-manno-D-*gala*-heptitol, XIV) obtained by the reduction of natural sedoheptulose (D-altroheptulose) was believed to be optically inactive; hence, incorrect configurations were assigned to it and to the 2-epimer α-sedoheptitol, the natural D-volemitol (XV). More recently it has been shown that (XIV) possesses a slight optical activity in water and a somewhat greater activity in borax.[72]

Borax enhances the rotation of polyols (see Chapter IV), but even the use of borax may not be a sufficiently dependable indication, e.g., for D-gala-L-*gala*-octitol (XXI).[77] The observed rotation of this polyol in water using sodium light was 0.0 and in borax it was only −0.5°.

Hudson has suggested conversion of polyols to the fully acetylated derivatives which in every known instance have pronounced rotations in chloroform when the configurations are not *meso*.

The use of ammonium molybdate solutions instead of borax may also be of value for the purpose.[72, 80] Some values reported by Frèrejacque[80] for mannitol are given in Table I.

The rotations in Table I are reported for $[\alpha]_{4861}$; those for $[\alpha]_D$ should be in the neighborhood of 80 or 85% of these values and hence well above 100°. Such rotations are very high for a polyol. It should also be noted that the rotation is practically constant for ratios between 2.84 and 12.85. Inasmuch as sorbitol and D-arabitol are reported to have rotations of $[\alpha]_{4861}$ +130° and +131°, respectively, it would be expected that the other optically active polyols would have rotations of the same order of magnitude.

Another method for the establishment of the configuration of a polyol

[79] F. B. LaForge, *J. Biol. Chem.*, **42**, 367, 375 (1920).

[80] M. Frèrejacque, *Compt. rend.*, **200**, 1410 (1935); **208**, 1123 (1939).

obtained synthetically is especially valuable when both of the 2-epimers
are optically active. It consists of the synthesis of the same polyol from
two different aldoses. Thus, from the fact that D-mannose and L-galactose
by the cyanohydrin synthesis yield four heptoses, which on reduction give
only three different heptitols, one of which (D-perseitol) is produced from
both D-mannose and L-galactose, the configuration of the four heptoses and
three heptitols could be deduced. (For a discussion of these methods and
detailed references, see Hudson.[60])

"α-Glucoheptulitol," obtained by Bertrand and Nitzberg[74] by the so-
dium amalgam reduction of L-glucoheptulose, which had been made by the
bacterial oxidation of gluco-*gulo*-heptitol (α-glucoheptitol) appears to be a
mixture of the optically inactive α-glucoheptitol and an optically active
compound.[81] Its enantiomorph is also known.[81]

TABLE I

*Rotations of D-Mannitol in Ammonium Molybdate Solution**

(1) Mannitol (millimoles)	(2) MoO₃ (millimoles)	Ratio $\frac{(2)}{(1)}$	
0.747	9.6	12.55	+168.5
0.861	4.8	5.57	+169.1
1.689	4.8	2.84	+168.8
2.298	4.8	2.10	+167.8
2.479	4.8	1.94	+164.4
2.716	4.8	1.76	+148.8

* Weighed amounts of D-mannitol were added to a solution of 5 ml. of N H₂SO₄
and 5 or 10 ml. of 0.1 N ammonium paramolybdate and diluted to 50 ml.

The fact that the melting point of the "D-glucoheptulitol" (141°) is
twelve degrees higher than that for α-glucoheptitol indicates that a sig-
nificant portion of the material consists of a second product. This substance
or impurity is not the epimeric L-gluco-D-*ido*-heptitol (L-β-glucoheptitol),
for a satisfactory separation of the epimers can be accomplished when L-
glucoheptulose is reduced catalytically.[82] That the amalgam reduction has
taken an unusual turn is indicated by the fact that when the product is
treated with boiling 0.1 N sulfuric acid or 10% barium hydroxide no
change results. If, however, 10% sulfuric acid is used, the rotation changes
sign, and α-glucoheptitol can be isolated. The possibility that "α-gluco-
heptulitol" may be an anhydride is unlikely because the elementary analy-
sis requires the unusual composition of four moles of anhydride to three of

[81] F. L. Humoller, S. J. Kuman and F. H. Snyder, *J. Am. Chem. Soc.*, *61*, 3370
(1939).
[82] Y. Khouvine, *Compt. rend.*, *204*, 983 (1937).

water, and because recrystallization from a variety of anhydrous solvents and heating at 100°C. under high vacuum failed to dislodge possible water of hydration. Evidently, further investigation is necessary.

The configurations of the known octitols have been established by means of the methods mentioned above.

For the nonitol and the decitol (XXII and XXIII) only the configuration of the hydroxyls corresponding to those of the preceding octose are known with certainty. However, an interesting regularity in the cyanohydrin synthesis[76, 83] permits the tentative assignment of configuration to the new hydroxyls resulting from application of this reaction to D-gluco-L-*gala*-octose. The generalization, which applies in the mannose, glucose, galactose and gulose series to the products obtained by the cyanohydrin synthesis, states that "the isomer which is more readily accessible is the one that carries its hydroxyls on carbon atoms 2 and 4 in *trans* relationship."[76]

The generalization may be applied to the tentative assignment of configurations to gluconononitol (XXII) and glucodecitol (XXIII). On the basis of this generalization, it may be predicted that a nonitol with the configuration (C) could be produced from D-mannose by successive applications of the cyanohydrin synthesis.
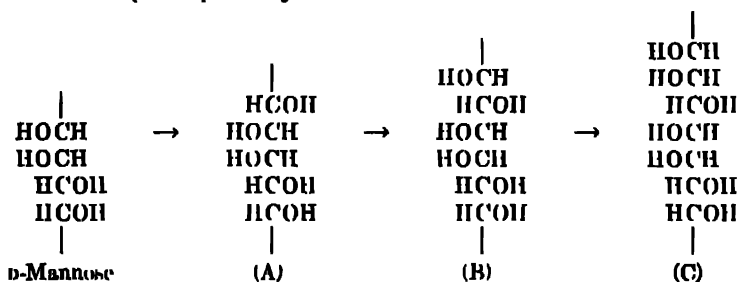
(A) represents the configuration of the principal product derived from D-mannose and (B) that of the minor product from A. (C), a major product from (B), should be identical with the known nonitol from D-glucose; if the nonitol were prepared in this manner from mannose and found to be identical with that from D-glucose, its configuration would be definitely fixed. The configuration of the glucodecitol could be determined in a similar manner with L-galactose as the starting product. Furthermore, the validity of the generalization could be checked at each step, for each of the intermediates or its enantiomorphous form is already known.

3. Synthesis

The classical method for the synthesis of polyols is based on the reduction of the corresponding aldoses or ketoses. Aldoses give one product and

83 C. S. Hudson, *Advances in Carbohydrate Chem.*, 1, 26 29 (1945).

ketoses two products, epimeric at carbon 2. Sodium amalgam and electrolytic reductions and catalytic hydrogenations have been employed, direct hydrogenation and electrolytic reduction being the commercially preferred methods (see under Sorbitol).

The cyanohydrin synthesis (p. 116) frequently is used as an intermediate step in the preparation of the higher-carbon polyols. The lactones and esters of the aldonic acids or other derivatives may be reduced directly to the corresponding glykitols. The reaction of diazomethane with aldonyl chlorides (p. 118) may be used for the same purpose particularly in instances when the desired product is a minor product of the cyanohydrin synthesis. These and other methods of increasing the length of the carbon chain are discussed in Chapter III.

Aminoglykitols, derived from nitroglykitols and from glycosylamines (Chapter IX), may be converted to polyols by treatment with nitrous acid, but anhydro rings may be formed (Chapter VIII).

The methods of total synthesis used in the preparation of pentitols and hexitols[20] [25] [46] [57] are of interest because of the departure from the methods of classical carbohydrate chemistry. Illustrations are given above under the pentitols and hexitols. The principal features of these syntheses are:

(1) The use of either the mono- or di-Grignard acetylide. If the mono-Grignard derivative of acetylene is reacted with an aldehyde, the resulting compound has a terminal ethynyl group. When the di-Grignard acetylide is used, a symmetrically substituted acetylene is obtained.

(2) The use of Bourguel's catalyst. This catalyst is colloidal palladium on starch, the starch being used as a protective colloid.[64] The reduction is carried out at room temperature. A sharp decrease in the rate of hydrogen consumption is noted when the acetylenic derivative has been converted to the ethylenic derivative. It is claimed that only the cis form of the ethylene derivative is obtained.

(3) The use of silver chlorate-osmic acid as the oxidizing agent. The osmic acid is present in catalytic amounts and probably acts as the oxygen carrier because at the completion of the reaction, when the chlorate is consumed, the osmic acid is reduced. The oxidation of the ethylenic double bond to the diol is carried out at room temperature for several days. A considerable portion of the ethylenic derivative must be oxidized in a different manner, for the yields of recovered polyol are low.

The scope of the synthesis could be extended by using aldehydo-sugars, probably as the acetates, for condensation with the acetylenic Grignard reagent. However, the configurations of the new polyols at the point of the original unsaturation would not be known without further investigation.

Biochemical syntheses of possible commercial value appear to be limited

[64] M. Bourguel, *Bull. soc. chim.*, [4] *45*, 1067 (1929).

to the production of D-mannitol and galactitol. High conversions of glucose and sucrose to mannitol are given by some species of *Aspergillus*.[41] It is believed that a microbiological Cannizzaro type reaction is involved, for gluconic acid is produced simultaneously. Certain bacilli are reported to give practically quantitative reductions of fructose to mannitol.[42] Galactitol can be extracted from the yeast, *Torula utilis*, grown on wood hydrolysates.[35]

4. Reactions

As the anhydrides, acetals and ketals of polyols are treated elsewhere in this text they will be omitted in the present discussion.

A. Esterification. The esterification methods used for the sugars (Chapter IV) are applicable to the polyols. The preparation and properties of organic and inorganic esters of polyols and their anhydrides are also considered in Chapter VIII. Fully esterified derivatives are generally unobtainable by direct esterification with organic acids because internal anhydrides are formed. Partial esters, especially di-esters, may be obtained by the use of amounts of benzoyl or *p*-toluenesulfonyl chlorides insufficient for complete esterification.

A number of inorganic esters are known. The nitrates are explosive and also may be used as vasodilators just as glycerol trinitrate is used.

Dichlorohydrins, as a rule, are readily obtained by direct reaction although some are easily converted to anhydro polyols. Higher halohydrins can only be obtained by indirect means. Thus, erythritol tetraacetate was converted to a tetrabromohydrin, m.p. 118°, by hydrobromic acid in glacial acetic acid at 150°C., whereas mannitol hexaacetate could not be carried beyond the pentabromo stage at 130-140°.[56] Mannitol was converted indirectly to a hexachlorohydrin by treatment of isomannide dichlorohydrin with fuming hydrochloric acid (see Chapter VIII).

Thionyl chloride, as well as other inorganic acid chlorides, reacts with polyols to form mixed esters (see under sulfate esters, Chapter IV). In the presence of pyridine, partial chlorohydrin formation may occur.[57]

Selenium oxychloride forms a selenite ester upon reaction with mannitol.[58] Phosphorus pentachloride yields unsaturated chlorohydrins of mannitol and galactitol which have the composition $C_6H_6Cl_4$.[59]

Extremely interesting are the so-called complexes of the glykitols with various inorganic polybasic acids, their salts or anhydrides in aqueou

[41] German Patent 700,919 (1940).

[56] W. H. Perkin, Jr , and J. L. Simonsen, *J. Chem. Soc.*, **87**, 855 (1905).

[57] Z. Kitasato and C. Sone, *Ber.*, **64**, 1142 (1931); R. Majima and H. Simanuki, *Proc. Imp. Acad. (Tokyo)*, **2**, 544 (1926).

[58] C. Chabrié and A. Bouchonnet, *Compt. rend.*, **136**, 376 (1903).

[59] J. C. Bell, *Ber.*, **12**, 1271 (1879).

solutions. "Complexes" with boric, molybdic, tungstic and other acids as well as the oxides of antimony and arsenic have been reported. It is believed that these complexes are true esters with one or more moles of polyol, a chelate type of structure being involved at some point. For the hexitols a compound with boric acid such as the following is postulated.[90]
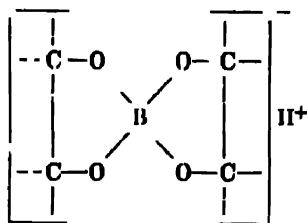
These compounds usually are known only in solution although some salts appear to have been obtained by precipitation of concentrated solutions with alcohol[91] (see also Chapter IV).

Some of the effects produced by adding such acids or salts to solutions of polyhydroxyl compounds are increased conductivity and acidity of the solution, exaltation of the rotation of optically active substances and marked changes of volume. For an example, see Table I, above.

Böeseken and his students have studied extensively the conductivity of solutions containing polyhydroxyl compounds and boric acid and have been able to apply the information thus obtained to the interpretation of the configuration of a number of compounds (see also Chapter II). The behavior of polyhydroxyl compounds is explained on the basis of a tendency for the repulsion of adjacent hydroxyl groups.[92] For open-chain α-glycols, the mutual repulsion of the hydroxyl groups with free rotation of the carbon atoms does not permit complex formation, and, hence, very little change in conductivity is noted. When the hydroxyls are in a ring compound, free rotation is not possible and cis α-hydroxyls have a greater tendency than trans to form complexes. The increase in conductivity obtained for acylic polyols, from diols to hexitols, is explained on the basis of decreased symmetry with respect to the hydroxyls and, therefore, a decrease in their repulsive action; consequently, there will be a greater opportunity for complex formation and, as a result, greater conductivity. Although the above generalizations can be used as a guide, too much reliance must not be placed on them, for many complex factors are involved and the

[90] H. Diehl, *Chem. Revs.*, *21*, 52 (1937).

[91] A. Grün and H. Nossowitch, *Monatsh.*, *37*, 409 (1916).

[92] J. Böeseken and J. M. Furnée, *Rec. trav. chim.*, *59*, 97 (1940); J. Böeseken, *Bull. soc. chim.*, [4] *53*, 1332 (1933).

results obtained by other workers are not completely in accord with the earlier work.[93]

The effect of mannitol on the acidity of boric acid is sufficiently great that the latter behaves like a strong monobasic acid and can be titrated directly; this observation is the basis of common methods for the determination of boric acid.

Two crystalline monoborate esters of mannitol and one dimetaborate ester are described. All have been obtained by reaction under practically anhydrous conditions. They are as follows:

Monoborate, m.p. 88.5 89.5°; $[\alpha]_D^{20}$ +15.1° (pyridine).[91][95] This product was obtained by reaction of the components in ethanol solution.

Monoborate, m.p. 79 80°; $[\alpha]_D^{20}$ +5.73° (pyridine).[95] This substance was obtained by heating a concentrated aqueous solution of the reactants at 120°C. until approximately two moles of water of reaction were driven off and crystallizing the resulting melt from water.

The first substance appears to be a mannitol 1-monoborate, whereas the second is a mannitol 2-monoborate.[95]

Mannitol dimetaborate,[96] according to its analysis, appears to be an addition compound of two moles of metaboric acid and one mole of mannitol. However, on benzoylation only mannitol 1,6-dibenzoate is obtained; hence, it is indicated that an ester-type linkage exists between the borate fragment and the mannitol. This borate was obtained by reaction of the components in anhydrous acetone. (Other products are described under borate esters in Chapter IV.) It has not yet been determined whether esterification occurs when mannitol and boric acid are dissolved together in water.

B. Oxidation.[97] With nitric acid, the polyols may be oxidized to the corresponding dibasic acids. This procedure provides a qualitative identification of galactitol by converting it to insoluble mucic acid, but galactose and galacturonic acid give the same product.

With other oxidizing agents, it is possible to obtain reducing sugars. Bromine water produces a mixture of the corresponding aldoses and 2-ketoses. Before the bacterial process was perfected, oxidation of sorbitol by bromine to sorbose was widely used in the laboratory.

Hydrogen peroxide in the presence of ferrous ions likewise forms reducing

[93] F. K. Bell, C. J. Carr, W. E. Evans and J C. Krantz, Jr., *J. Phys. Chem*, *42*, 507 (1938).

[94] J. J. Fox and A. J. H. Gauge, *J. Chem. Soc.*, *99*, 1075 (1911).

[95] W. H. Holst, Paper presented before the Division of Sugar Chemistry and Technology, Am. Chem. Soc., April (1939)

[96] P. Brigl and H. Gruner, *Ann.*, *495*, 70, 72 (1932).

[97] See Chapter VII for additional details.

sugars from polyols.[98] Erythritol, mannitol, galactitol and sorbitol were oxidized in this manner, and either the free sugars or the osazones were isolated.

Platinum, apparently acting as a carrier for oxygen, oxidizes polyhydric alcohols to reducing sugars and sugar acids.[99] In the presence of a hydrogen acceptor such as quinone, sunlight causes the dehydrogenation of polyols to the corresponding aldoses.[100] Potassium ferricyanide in a modified Hagedorn-Jensen procedure is capable of oxidizing polyols. However, the nature of the oxidation products was not ascertained.[101]

A number of polyols was oxidized electrolytically at platinum electrodes in the absence of an electrolyte.[102] Aldoses and ketoses were formed and isolated as osazones or hydrazones. Acids also were formed, and, with erythritol, a keto acid was produced. When the oxidation was carried out in the presence of sodium bromide using carbon electrodes, ketoses were obtained free of degradation products.[103]

Sodium chlorite appears to attack polyols rather slowly in comparison to aldoses, at least in the case of mannitol.[104]

Oxidation of polyols by microorganisms is usually the best method for their conversion to ketoses. The yields are high, and the process is the preferred commercial one for the conversion of sorbitol to L-sorbose, an intermediate in the production of vitamin C. Additional information is given elsewhere in this text (Chapter III, synthesis of ketoses).

C. Reduction. Reduction in the glykitol series results in conversion to desoxy derivatives or hydrocarbons, for the only groups present are alcoholic hydroxyls. The reduction of glykitols to secondary alkyl iodides by treatment with hydriodic acid according to the method of Erlenmeyer and Wanklyn[105] is mainly of historical interest. Until the LeBel and Van't Hoff theory of the asymmetric carbon atom was proposed, the difference between mannitol and dulcitol and between the alkyl iodides derived therefrom was unclear and the subject of much controversy. To explain the slight differences in the hexitols and in the reduction products, it was suggested that galactitol had a branched carbon skeleton, a concept that became unnecessary when the theory of the asymmetric carbon atom was accepted.

[98] H. J. H. Fenton and H. Jackson, *J. Chem. Soc.*, **75**, 1 (1899).

[99] J. W. E. Glattfeld and S. Gershon, *J. Am. Chem. Soc.*, **60**, 2013 (1938); E. von Gorup-Besanez, *Ann.*, **118**, 257 (1861).

[100] G. Ciamician and P. Silber, *Atti acad. Lincei*, [5] **10**, I, 92 (1901).

[101] W. R. Todd, J. Vreeland, J. Myers and E. S. West, *J. Biol. Chem.*, **137**, 269 (1939).

[102] C. Neuberg, *Biochem. Z.*, **17**, 270 (1909).

[103] J. E. Hunter, *Iowa State Coll. J. Sci.*, **15**, 78 (1940)

[104] A. Jeanes and H. S. Isbell, *J. Research Natl. Bur. Standards*, **27**, 125 (1941).

[105] E. Erlenmeyer and J. A. Wanklyn, *Ann.*, **135**, 129 (1865).

Degradative reduction of polyols has not been investigated for very many substances. Sorbitol and glucose, which gives sorbitol under reducing conditions, have been converted to lower polyhydric alcohols by subjection to high temperatures and pressures and the action of oxides of copper and aluminum.[106] Propylene glycol, glycerol and polyols of higher molecular weights were obtained. It would be expected that all the higher polyols are capable of this type of degradative reduction.

D. Etherification. Etherification procedures for glykitols are the same as those for the other carbohydrates (Chapter VIII). Sometimes, however, the attainment of fully etherified products may be difficult. Mannitol, for example could not be converted to the hexamethyl ether despite repeated treatment with methyl iodide and silver oxide[107] or with methyl sulfate and alkali.[108]

E. Qualitative and Quantitative Determination. Because of the closely similar chemical behavior of the polyols, the analysis of polyols is difficult. However, there are a few specific reactions that serve to identify or quantitatively to determine some of the polyols. Among the specific reactions is the oxidation of dulcitol to insoluble mucic acid. Sorbitol forms a relatively insoluble pyridine complex;[42] as far as known, only 2-desoxysorbitol (2-desoxymannitol) forms an analogous molecular complex.[109] The complex formed from 2-desoxysorbitol and pyridine appears to be more soluble in pyridine than that from sorbitol, but both are readily decomposed by small amounts of water.

If the nature of the polyol is known, and there are no interfering substances, quantitative determinations are sometimes possible. Thus, mannitol can be determined polarimetrically by means of its borax complex even in the presence of sorbitol.[110] An empirical oxidation with potassium ferricyanide (modified Hagedorn-Jensen method) has been devised for the determination of sorbitol, mannitol, galactitol, erythritol and other polyhydric substances.[101] The number of hydroxyls per mole can be determined by various acylation procedures or by periodate or lead tetraacetate oxidation (see Chapter VII).

Adulteration of grape wine by the addition of other fruit wines may be detected because of the presence of sorbitol in the latter. Litterscheid's o-chlorobenzaldehyde method appears to be the best of the various tests proposed. However, mannitol may interfere if present in excessive amounts.[111]

[106] C. W. Lenth and R N. DuPuis, *Ind Eng. Chem.*, **37**, 152 (1945).

[107] J. C. Irvine and B M. Paterson, *J. Chem. Soc.*, **105**, 915 (1914).

[108] W. N. Haworth, *J. Chem. Soc.*, **107**, 10 (1915).

[109] M. L. Wolfrom, M. Konigsberg, F B. Moody and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **68**, 122 (1946).

[110] R. S. Rose, Jr and R. M. Goepp, Jr , Abstracts of the Div. of Sugar Chemistry and Technology, The American Chemical Society, April (1939).

[111] J Jeanprêtre, *Mill Lebensm Hyg.*, **28**, 87 (1937).

F. Biochemistry.[2] None of the polyols appears to have any specific fundamental physiological significance except ribitol (adonitol) which is a component of vitamin B₂, riboflavin (see Chapter IX). If fed in sufficiently large amounts to rats, polyols have some narcotic effect.[112] The amount required increases in general with the number of hydroxyls, ranging from 80 mg. of ethanol, per 100 g. of body weight, to over 380 mg. of perseitol and volemitol. The minimal dosage of dulcitol is 120 mg. and of mannitol is 320 mg.

Of the polyols studied, only sorbitol and D-mannitol appear capable of being partially or completely utilized by the human body, but they are not the equivalent of D-glucose. Although sorbitol can be a precursor of blood carbohydrate under certain conditions, apparently it is preferentially stored in the liver as glycogen, and the blood sugar level is not noticeably increased. For this reason, sorbitol may have value as a sweetening agent in the diabetic diet.

Although the polyols are not considered to be toxic, large doses taken orally may cause severe diarrhea. Solutions of glykitols are capable of causing hemolysis of red blood cells; this appears to be an osmotic effect, and the time required for a given degree of hemolysis increases with the molecular weight of the polyol.[113]

As a class, the polyols appear to be capable of behaving as nutritive substrates for a large variety of microorganisms, but no single organism appears capable of utilizing every polyol. D-Mannitol seems to be more generally utilizable than sorbitol, whereas for the related sugars D-glucose is attacked more readily than D-mannose.

In the higher plants and particularly the fruits the polyols appear to function as reserve carbohydrate, the amount present being seasonal and becoming less as the sugars increase during the ripening process.

In taste the polyols range from faintly sweet to extremely sweet, the threshold value for erythritol being considerably less than for sucrose.[114]

5. Desoxy Polyols

A class of acyclic polyols not yet mentioned is that in which the number of carbon atoms is greater than the number of hydroxyl groups. These compounds may be subdivided on the basis of the number of carbon atoms or of the number of hydroxyls. The generally preferred classification is the former in which case the prefix desoxy is coupled with a descriptive name indicating the configuration of the related fully hydroxylated polyol, e.g., 1-desoxy-D-galactitol

[112] D. I. Macht and G. C. Ting, *Am. J Physiol.*, 60, 196 (1922)

[113] A. M. Kunkel, C. J Carr and J. C. Krantz, Jr., *Proc. Soc. Exptl. Biol. Med.*, 42, 438 (1939).

[114] C. J. Carr, F Frances and J. C. Krantz, Jr., *J. Am. Chem. Soc.*, 58, 1394 (1936)

All terminal monodesoxy polyols can be given more than one descriptive name because either of the penultimate asymmetric carbon atoms can be used as the basis of the D.L classification. The above compound can also be called 6-desoxy-L-galactitol. It is proposed (and will be followed in this section) that all terminal monodesoxy compounds be named with the desoxy carbon in the 1-position. This procedure reverses the manner in which the earlier desoxy polyols, derived from terminal-desoxy hexoses, have been written. However, in view of the development of synthetic procedures for the conversion of aldehydic carbon atoms to the desoxy state, the convention is based on a more generally valid genetic relationship than the older one based on the naturally occurring terminal desoxy sugars.

For the 2-desoxypolyols, which can be considered related to more than one polyol (2-desoxy-sorbitol or -mannitol, for example), the nomenclature
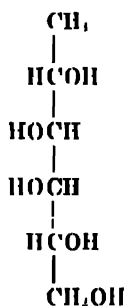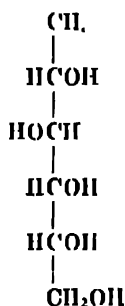
CH₃	CH₃	CH₃
HCOH	HCOH	HOCH
HOCH	HOCH	HCOH
HOCH	HCOH	HCOH
HCOH	HCOH	HCOH
CH₂OH	CH₂OH	CH₂OH
(XXIV)	(XXV)	(XXVI)
1-Desoxy-D-galactitol	1-Desoxy-D-glucitol	1-Desoxy-D-altritol
(L-fucitol)	(L-gulomethylitol)	(D-epifucitol)
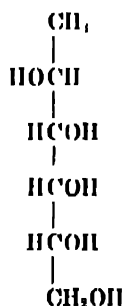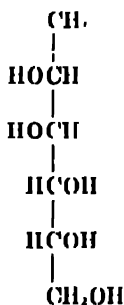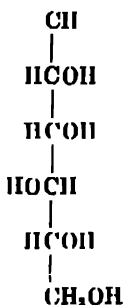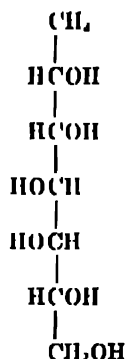
CH₃	CH₃	CH₃
HOCH	HCOH	HCOH
HOCH	HCOH	HCOH
HCOH	HOCH	HOCH
HCOH	HCOH	HOCH
CH₂OH	CH₂OH	HCOH
(XXVII)	(XXVIII)	CH₂OH
1-Desoxy-D-mannitol	1-Desoxy-D-gulitol	(XXIX)
(D-rhamnitol)	(L-epirhamnitol)	1-Desoxy-D-gala-L-*manno* heptitol
		(L-α-rhamnohexitol)

of Sowden and of Wolfrom, Thompson and Evans[115] may be used. According to this system 2-desoxysorbitol may be called 2-desoxy-D-*arabo*-hexitol.

With the exception of the 1,4-didesoxy tetritols (2,3-butylene glycols), which can be obtained by a fermentation process, none of the other mono- and di-desoxy polyols have been found in nature. The D-configurations of the known 1- and 2-monodesoxy polyols are given in formulas XXIV to XXXII.

CH₃
|
HCOH
|
HOCH
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH

(XXX)
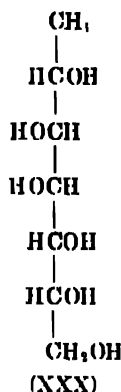
1-Desoxy-D manno D-*gala* heptitol[a]

(L-α fucoheritol)

CH₃
|
HCOH
|
HCOH
|
HOCH
|
HOCH
|
HOCH
|
CH₂OH

(XXXI)

1 Desoxy-L-altro-L-*manno*-heptitol[b]

(L-β-rhamnoheritol)

CH₂OH
|
CH₂
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH

(XXXII)
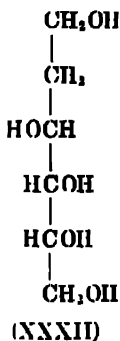
2 Desoxy D *arabo* hexitol

(2-desoxy D sorbitol or D mannitol)

A. Synthesis and Properties of Monodesoxy Polyols. 1-Desoxy-D-galactitol (L-fucitol) (XXIV), m.p. 153-4°; $[\alpha]_D^{20} + 4.7°$ (borax); pentaacetate, m.p. 127°; $[\alpha]_D^{25} + 20.5°$ (CHCl₃). This compound has been prepared by

[a] Probable configuration

[b] The D-enantiomorph is not known

[115] J. C. Sowden, *J. Am. Chem. Soc*, **69**, 1047 (1947), M. L. Wolfrom, A. Thompson and E. F. Evans, *ibid.*, 1794 (1945)

the sodium amalgam reduction of natural L-fucose[116] and by the demercaptalation of D-galactose diethyl mercaptal by massive amounts of Raney nickel.[117]

1-Desoxy-L-galactitol (D-fucitol, D-rhodeitol), m.p. 153.5°; $[\alpha]_D - 1.45°$ (H_2O); it was synthesized by the reduction of D-fucose.[118]

D,L-1-Desoxygalactitol (D,L-fucitol), m.p. 168°, was prepared by the sodium amalgam reduction of D,L-fucose.[118]

1-Desoxy-D-glucitol (1-desoxysorbitol, L-gulomethyltol) (XXV), m.p. 131-2°; $[\alpha]_D^{20} + 3.95 \pm 1.5°$ (H_2O), $+ 16.7°$ (sat. borax); pentaacetate, m.p. 105-6°; $[\alpha]_D^{21} + 21°$ (CH_3OH). This polyol was obtained by the reduction of 6-desoxy-L-gulonic acid[119] which was synthesized from 1,2-isopropylidene-L-xylofuranose and also from diisopropylidene-aldehydo-D-arabinose by use of the Grignard reagent.[120]

1-Desoxy-D-altritol (D-epifucitol, 6-desoxy-D-talitol) (XXVI) was synthesized by the reduction of 6-desoxy-D-talose.[121] It melts at 104°, $[\alpha]_D + 2°$ (H_2O); dibenzylidene derivative, m.p. 184°, $[\alpha]_D - 40.9°$ ($CHCl_3$). However, a melting point of 110–111° is reported for the polyol obtained by the reduction of epimerized D-fuconic acid.[120]

1-Desoxy-L-altritol (L-epifucitol), m.p. 104, $[\alpha]_D - 2.3°$ (H_2O); dibenzylidene derivative, m.p. 183°, $[\alpha]_D + 39.7°$ ($CHCl_3$). This product was synthesized by the reduction of 6-desoxy-L-talonic acid.[122]

1-Desoxy-D-mannitol (D-rhamnitol) (XXVII), m.p 123°, $[\alpha]_D - 12.4°$ (H_2O); trihydrate, m.p. 69°; dibenzylidene derivative, m.p. 207°, $[\alpha]_D + 60.7°$ ($CHCl_3$). The polyol was synthesized by the reduction of D-rhamnose[123, 124] and by the reduction of the reaction product of diisopropylidene-aldehydo-D-arabinose and methyl magnesium iodide;[120] the epimeric 1-desoxy-D-glucitol was produced simultaneously

1-Desoxy-L-mannitol (L-rhamnitol). (The constants are the same as for the enantiomorph except for the opposite sign of rotation.) This substance has been prepared by the sodium amalgam reduction of naturally occurring L-rhamnose[123] and by the pressure hydrogenation of L-rhamnonic lactone.[40]

1-Desoxy-D-gulitol (L-epirhamnitol, 6-desoxy-L-glucitol) (XXVIII), sirup, $[\alpha]_D^{20} + 9.18°$ (H_2O); dibenzylidene derivative, m.p. 193-4°, $[\alpha]_D^{20} +$

[116] E. Votoček and R. Potměšil. Ber., 46, 3053 (1913).
[117] M. L. Wolfrom and J. V. Karabinos, J. Am. Chem. Soc., 66, 909 (1944).
[118] E. Votoček and J. Bulíř, Chem. Zentr., 77, 1, 1818 (1906).
[119] H. Müller and T. Reichstein, Helv. Chim. Acta, 21, 251 (1938).
[120] K. Gätzi and T. Reichstein, Helv. Chim. Acta, 21, 914 (1938).
[121] E. Votoček and F. Valentin, Collection Czechoslov. Chem. Commun., 2, 36 (1930).
[122] E. Votoček and V. Kučerenko, Collection Czechoslov. Chem. Commun., 2, 47 (1930).
[123] E. Votoček, F Valentin and F. Rác, Collection Czechoslov. Chem. Commun., 2, 402 (1930).
[124] F. Valentin, Collection Czechoslov. Chem. Commun., 2, 689 (1930).

32.3° (CHCl₃); second dibenzylidene derivative, m.p. 196°, $[\alpha]_D^{20}$ − 36.7° (CHCl₃). This desoxy polyol was synthesized by the reduction of 6-desoxy-L-gluconic lactone.[12b]

1-Desoxy-L-gulitol (D-epirhamnitol, 6-desoxy-D-glucitol, 6-desoxysorbitol) sirup, $[\alpha]_D^{20}$ − 10.0° (H₂O); dibenzylidene derivative, m.p. 196°, $[\alpha]_D^{20}$ + 36.7° (CHCl₃). This product was synthesized by the sodium amalgam reduction [126] and by the pressure hydrogenation of 6-desoxy-D-glucose.[127]

1-Desoxy-D-gala-L-manno-heptitol (L-α-rhamnohexitol) (XXIX), m.p. 180–3°, $|\alpha|_D$ + 11°; monotrityl ether, m.p. 103–4°, $|\alpha|_D$ − 2.0° (methanol and benzene). This product was synthesized by the reduction of the corresponding lactone.[12b]

1-Desoxy-D-manno-D-gala-heptitol (L-α-fucohexitol) (XXX), m.p. 179-80°, $|\alpha|_D$ + 0.3° (H₂O), + 1.7° (sat. borax); no crystalline benzylidene derivative known. The polyol was synthesized by the sodium amalgam reduction of the corresponding sugar.[121]

1-Desoxy-L altro-L-manno-heptitol (L-β-rhamnohexitol) (XXXI), sirup; monobenzylidene derivative, m.p. 233–4°, $|\alpha|_D$ + 59.8° (CHCl₃). This glykitol was synthesized by the reduction of the corresponding lactone.[128]

2-Desoxy-D-arabo-hexitol (2-desoxy-D-mannitol or -D-sorbitol) (XXXII), m.p. 105–6°, $[\alpha]_D^{23}$ + 17.5° (H₂O), $[\alpha]_D^{28}$ + 35° (borax); pentaacetate, m.p. 86 7°, $[\alpha]_D^{21}$ + 35.3° (CHCl₃). This product has been synthesized by the reduction of 2-desoxy-D-glucose,[146] and the pentaacetate was synthesized by desulfurization of keto-D-fructose pentaacetate diethyl mercaptal.[117]

1-Desoxyheptitol of unknown configuration, m.p. 189–90°; hexaacetate, m.p. 142°. This was synthesized by the oxidation with silver chlorate and osmium tetroxide of vinyl propenyl glycol obtained by reduction (zinc-copper couple) of a mixture of acrolein and crotonaldehyde.[55]

B. Synthesis and Properties of Didesoxy Polyols. The formulas of the didesoxy polyols of known configuration are given in formulas XXXIII to XXXVI.

1,4-Didesoxyerythritol (meso-2,3-butylene glycol) (XXXIII), m.p. 25°; pentahydrate, m.p. 16.8°. This compound has been obtained by the fermentation of glucose with *Aerobacter aerogenes*.[131] About 10 per cent of the dextrorotatory 1,4-didesoxy-L-(d)-threitol is also obtained.

[12b] E. Votoček and J. Mikšič, *Chem. Abst.*, **22**, 2740 (1928).

[126] E. Votoček and F. Rác, *Collection Czechoslov. Chem. Commun.*, **1**, 239 (1929).

[127] A. T. Ness, R. M. Haun and C. S. Hudson, *J. Am. Chem Soc.*, **66**, 1236 (1944).

[128] E. Fischer and O. Piloty, *Ber.*, **23**, 3106 (1890); F. Valentin, *Collection Czechoslov. Chem. Commun.*, **3**, 499 (1931).

[121] E. Votoček and F. Valentin, *Coll. Czechoslov. Chem. Commun.*, **10**, 77 (1938).

[146] M. L. Wolfrom, M. Konigsberg, F. B. Moody and R. M. Goepp. Jr , *J. Am. Chem. Soc.*, **68**, 124 (1946).

[131] G. E. Ward, G. G. Pettijohn, L. B. Lockwood and R. D. Coghill, *J Am Chem Soc.*, **66**, 541 (1944).

$$
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{HCOH} \\
| \\
\text{HCOH} \\
| \\
\text{CH}_3 \\
\text{(XXXIII)}
\end{array}
\qquad
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{HOCH} \\
| \\
\text{HCOH} \\
| \\
\text{CH}_3 \\
\text{(XXXIV)}
\end{array}
$$

1,4-Didesoxyerythritol   1,4-Didesoxy-D-threitol

$$
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{HCOH} \\
| \\
\text{HOCH} \\
| \\
\text{HOCH} \\
| \\
\text{HCOH} \\
| \\
\text{CH}_3 \\
\text{(XXXV)}
\end{array}
\qquad
\begin{array}{c}
\text{CH}_3 \\
| \\
\text{HOCH} \\
| \\
\text{HOCH} \\
| \\
\text{HOCH} \\
| \\
\text{CH} \\
| \\
\text{CH}_2\text{OH} \\
\text{(XXXVI)}
\end{array}
$$
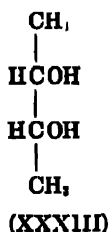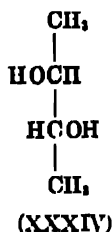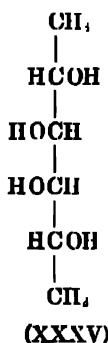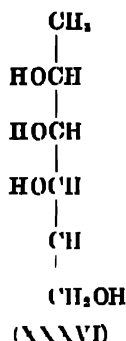
1,6-Didesoxygalactitol   1,5-Didesoxy-L-ribo-hexitol
(Digitoxitol)

1,4-Didesoxy-D-threitol (*l*-2,3-butylene glycol) (XXXIV), m.p. 19°, $[\alpha]_D^{25} - 13.0°(\text{H}_2\text{O})$. This diol is produced by *Bacillus polymyxa* when grown on grain mash.[141] A very small amount of the *meso*-isomer (XXXIII) is simultaneously produced.

1,6-Didesoxygalactitol (XXXV), m.p. 183 4°; diisopropylidene derivative, m.p. 62–63.5°. This polyol was obtained from L-fucitol and from galactitol by use of the tosyl-iodide exchange reaction.[132]

1,5-Didesoxy-L-ribo-hexitol (XXXVI) m.p. 88°; dibenzylidene derivative, m.p. 142°. This tetrol was obtained by reduction of the naturally occurring digitoxose.[141]

In addition to the above compounds two 1,6-didesoxyoctitols of unknown configuration are described. One melts at 161 2 and its hexaacetate melts at 108.5°, whereas the other melts at 250° and has a hexaacetate with a melting point of 159°. These products were prepared by the oxidation of dipropenyl glycol.[15]

In addition to the obvious reduction of related desoxy sugars and acids, a number of synthetic procedures are useful for the synthesis of desoxy polyols. The most common involves tosylation followed by conversion to

[132] A. T. Ness, R M. Hann and C. S. Hudson, *J. Am. Chem Soc*, **64**, 982 (1942).
[133] F. Micheel, *Ber.*, **63**, 347 (1930).

the iodide and then reduction (as under Tosyl esters, Chapter IV). The
compounds obtained in this manner contain the same number of carbon
atoms as the original substance. The oxidation of unsaturated glycols as
employed by Wiemann[58] (see above) constitutes a total synthesis. Here,
however, the configuration of the compounds formed in general will be
unknown. The various methods of lengthening the carbon chain can be used
with desoxy sugars as the starting products.

The desulfurization of *aldehydo* and *keto* mercaptals[117] is a general reaction
for the production of 1- and 2-desoxy polyols and deserves special mention.

C. Proofs of Structure and Configuration. For most of the desoxy
polyols, the structures and configurations are very simply related to the
parent sugar. In the case of the 1-desoxy-D-manno-D-*gala*-heptitol (XXX)
the configuration has not been unequivocally established because the con-
figuration of carbon 2 of the parent aldose is based on the empirical "amide
rule."

In the case of didesoxy-D-threitol (XXXIV) the probable configuration
is that indicated. Morell and Auernheimer[134] showed that didesoxy-L-
threitol is related to the dextrorotatory 2-butanol which had earlier been
related to L(d)-lactic acid. However, since considerable racemization oc-
curred in the transition from the butylene glycol to 2-butanol, some ques-
tion regarding the validity of this proof remains. It might be possible to
oxidize, without racemization, the optically active 2-butanol-3-one (ace-
tylmethyl carbinol) obtained by the fermentative oxidation of *meso*-butyl-
ene glycol to lactic acid. This procedure would then establish the absolute
configuration of the 2-butanol-3-one. Inasmuch as reduction of the 2-
butanol-3-one can produce only *one* optically active didesoxythreitol in
addition to didesoxy-*meso*-erythritol, the didesoxythreitol would be genet-
ically related to the lactic acid obtained by oxidation.

<div align="center">PART II</div>

<div align="center">THE INOSITOLS AND RELATED COMPOUNDS[135]</div>

In view of the wide distribution of cyclohexanehexols (called inositols or
cyclitols) and the importance of one in particular (*meso*-inositol) to certain
bacteria, plants and perhaps even to warm-blooded animals, the naturally
occurring and synthetic compounds of this carbocyclic type have received
considerable study.

Naturally occurring members include four inositols, monomethyl ethers,
monodesoxy derivatives, a methyl homolog, and desoxy carboxylic acids.
The synthetic members include two related ketones (cycloses), two inosi-
tols, a methyl homolog, and two hydroxymethyl inositols.

[134] S. A. Morell and A. H. Auernheimer, *J. Am. Chem. Soc.*, **66**, 792 (1944)

[135] H. G. Fletcher, Jr., *Advances in Carbohydrate Chem.*, **3**, 45 (1947).

The inositols, themselves, are typically crystalline, water-soluble, high melting compounds having a sweet taste. As alcohols, their reactions are similar to those of the acyclic polyols, but they differ in their behavior to strong nitric acid and to strong hydriodic acid which produce benzenoid compounds.

1. Isomerization and Representation of Configuration

Because of the cyclic structure and hence the great opportunity for molecular symmetry, the number of *meso* forms among the cyclohexane-hexols is greater than of the optically active forms. This is the reverse of the situation existing in the acyclic hexanehexol series.

Of the nine possible cyclohexanehexols, shown configurationally below

(I)   (II)   (III)   (IV)
                Alloinositol   *meso*-Inositol

(V)   (VI)   (VII)   (VIII)   (IX)
       Mucoinositol   *l*-Inositol   *d*-Inositol   Scyllitol
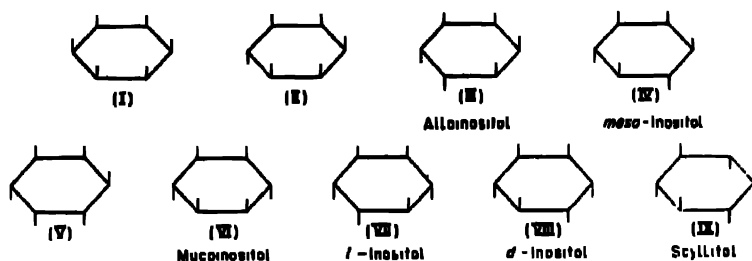
only two, (VII) and (VIII), are optical enantiomorphs. The remaining seven are optically inactive as a result of internal compensation and hence, technically, are all *meso* forms. However, the designation *meso* has been limited to one compound only (structure IV).

The *meso*-inositol is the most widespread of the four naturally occurring isomers (*d*- and *l*-inositol, and scyllitol being the others). It is sometimes called *i*-inositol (inactive) and more often just inositol. Here, it will be referred to as *meso*-inositol.

A systematic nomenclature based on configurations relating the cyclitols to the ordinary carbohydrates is not yet possible since the biochemical relationship of the cyclitols to the naturally occurring sugars has not been established. Accordingly, no consistent convention has been adopted for representing the cyclitols of known structure, although perspective projection is commonly employed. It is customary to show hydroxyl groups by vertical lines, and to omit the hydrogen atoms. The system is somewhat similar to that for Haworth formulas (p. 51). A numerical system is sometimes used to indicate orientation above and below the ring, thus,

$\frac{1, 4, 5}{2, 3, 6}$ denotes one of the two optically active inositols, but there is no established convention for numbering the carbons.

Since shikimic acid has been related configurationally to 2-desoxy-

gluconic acid, and since 2-desoxy sugars are encountered in nature, it is mnemonically useful to represent the cyclitols of known configuration so that quercitol becomes a 2-desoxy compound, and the other compounds follow the familiar *gluco-* and *manno-*configurational pattern as closely as

TABLE II

Mnemonic Arrangement for Common Cyclitols

Name	Proposed Mnemonic Orientation	Configurational Terminology	Customary Orientation
l-Inositol		D *gluco-trans-*1,2,6	
d-Inositol		L *gluco* trans-1,2,6	
d-Quercitol		2-desoxy D-*gluco* trans-2,6,1	
Dihydroconduritol		1,2-didesoxy D *gluco-trans* 2,6	
Mucoinositol		D *gluco* trans-2,6,1	
Alloinositol		D *manno* cis-1,2,6-	
Mannocyclitol		1,6 didesoxy D *manno*	
meso-Inositol		D *gluco* trans 2,1,6	
Scyllitol		L *ido* trans 2,1,6	
Dihydroshikimic acid	COOH	2,6 didesoxy D *gluco*	COOH

possible. The above table shows the common cyclitols arranged in this way with carbon 1 at the right (Table II)

Because carbon atom 6 is not asymmetric in hexose sugars and because the α,β-designations are not particularly suitable in this series, the configurations of carbon atoms 1 and 6 are related to that at 2 by the usual *cis*, *trans* system.

It will be noted that the first six compounds have identical configurations at points 3, 4, 5 and 6 except d-inositol which is inverted and that all the naturally occurring compounds are *trans* at 1, 2 or 2, 1, 6 and that the two synthetic inositols, allo- and muco-, are *cis* at 1, 2. Also, *meso*-inositol and scyllitol, which are related through a common 5-ketone, have the D-*gluco* and L-*ido* designation. Finally, dihydroshikimic acid,[1] which is configurationally related to 2-desoxygluconic acid, has the extra carboxyl at carbon 1 and the common D-*gluco* configuration at 3, 4 and 5. Figures 1 and 2 depict the genetic relationships among the cyclitols.

2. Occurrence and Synthesis

d-Inositol (β-inositol, matezodambose), m.p. 217–248°, $[\alpha]_D$ +65° (H_2O), occurs as the monomethyl ether, pinitol, m.p. 186°, $[\alpha]_D$ +65.5° (H_2O), in various conifers, particularly the sugar pine (*Pinus lambertiana*) and also in Madagascar rubber latex and in senna leaves.[2] Pinitol is sweet, very soluble in water, stable in dilute acids and alkalies, and oxidized by ammoniacal silver nitrate. Hydriodic acid at the boiling point (127°) demethylates the compound to d-inositol.

l-Inositol, the optical enantiomorph of d-inositol, likewise occurs as a monomethyl ether, quebrachitol, which is not the enantiomorph of pinitol. It is obtained by demethylation of quebrachitol with hydriodic acid. Quebrachitol, m.p. 190–191°, $[\alpha]_D$ −80.2° (H_2O), is found in quebracho bark and in the latex of *Hevea brasiliensis*.[2]

d,l-Inositol, m.p. 253°, is found in the free state in mistletoe berries and in blackberries.[2]

meso-Inositol (dambose, phascomannite, i-inositol), more often termed simply as inositol, m.p. 225-7°, is the most common of the group, being found in microorganisms, plants and animals. In plants it is generally present as phytin,[3] a calcium-magnesium salt of phytic acid,[4] the hexaphosphate ester of *meso*-inositol. Other lower phosphates are likewise encountered[5] whose formation is due to the action of a phosphatase, phytase.[6] *meso*-Inositol occurs both free and combined in muscle[7] and in the heart, lungs, liver and other parts of the animal body and body fluids. It is

[1] It is noteworthy that only one of the two possible epimers has been obtained by reduction of shikimic acid.

[2] "Beilsteins Handbuch der organischen Chemie," vol. *6*, p. 1193; J. Springer, Berlin (1923).

[3] *Ibid* , p. 1194.
M. Kobel and C. Neuberg, Klein's "Handbuch der Pflanzenanalyse" vol. 2, p. 570, (1932); also, C. Wehmer, W. Thies and M. Haddors, *ibid.*, p. 577.

[4] S. Posternak, *Compt. rend.*, *169*, 139 (1919); *Helv. Chim. Acta*, *4*, 150 (1921).

[5] R. J. Anderson, *J. Biol. Chem.*, *18*, 441 (1914); *20*, 465, 498 (1915); E. Klenk and R. Sakai, *Z. physiol. Chem.*, *258*, 33 (1939).

[6] R. J. Anderson, *J. Biol. Chem.*, *20*, 480, 485 (1915).

[7] J. Scherer, *Ann.*, *73*, 322 (1850).

Fig 1 — Cyclitols of the *d*- and *l* inositol group

Fig 2 — Cyclitols of the *meso*-inositol group

present to the extent of 6.8 to 8.6% in the phosphatide of brain cephalin or about 0.4% of the net weight of the brain.[5] In certain bacterial phosphatides it is built into a polysaccharide.[6]

Corn steep liquor provides a good source of phytin, precipitated as the calcium salt.[10a] meso-Inositol is prepared industrially from phytic acid by alkaline hydrolysis at elevated temperature and pressure. Complete agreement on the structure of phytic acid and its salts has not been reached.[10b] meso-Inositol has been synthesized by the hydrogenation of hexahydroxybenzene.[11]

Two monomethyl ethers of meso-inositol are known, bornesitol, m.p. 199°, from Borneo rubber, and sequoyitol,[12] m.p. 234 235°, in California redwood. A dimethyl ether, dambonitol, m.p. 195°, is found in Gabon rubber.[13] Bornesitol is optically active, whereas the other two methyl ethers are inactive.

Scyllitol (cocositol, quercin, m.p. 352°) the fourth known naturally occurring cyclohexanehexol, though not abundant, is widely distributed being found in the elasmobranch fishes (sharks, rays, dogfish),[14] in the dogwood,[15] in the leaves of the cocos palm[16] and in acorns.[17] It can be obtained synthetically from bioinosose[18] (see Fig. 2 and below).

d-Quercitol, m.p. 235-237°, $[\alpha]_D^{20} + 25.6°$, is the most common of the desoxy inositols and occurs in all parts of the oak, particularly in the acorn,[19] and in the leaves of European palm, Chaemcrops humilis.[20]

It is curious that d-quercitol and scyllitol, which are not configurationally related, should occur together in the acorn. d-Quercitol has been characterized as the pentabenzoate,[21] m.p. 155°; $[\alpha]_D^{23} + 61.4°$

Another optically active cyclohexanepentol, m.p. 171°, was discovered in the leaves of Gymnema sylvestre, a milk weed. It was given the name l-

[5] J. Folch and D. W. Woolley, J. Biol. Chem., 142, 963 (1942).

[6] J. Cason and R. J. Anderson, J. Biol. Chem., 126, 532 (1938).

[10a] See: E. Bartow and W. W. Walker, Ind. Eng. Chem., 30, 300 (1938); U. S. Patent 2,112,553, March 29, 1938; F. A. Hoglan and E. Bartow, Ind. Eng. Chem., 31, 749 (1939).

[10b] See, for example: R. J. Anderson, J. Biol. Chem., 17, 171 (1914); 44, 429 (1920); C. Neuberg, Biochem. J., 9, 557 (1908); S. Posternak, Compt. rend., 169, 37 (1919).

[11] H. Wieland and R. S. Wishart, Ber., 47, 2082 (1914).

[12] E. C. Sherrard and E. F. Kurth, J. Am. Chem. Soc., 51, 3139 (1929).

[13] A. Girard, Compt. rend., 67, 820 (1868).

[14] G. Staedeler and F. J. Frerichs, J. prakt. Chem., [1], 73, 48 (1858).

[15] R. M. Hann and C. E. Sando, J. Biol. Chem., 68, 399, (1926).

[16] H. Muller, J. Chem. Soc., 91, 1768 (1907); 101, 2383 (1912).

[17] C. Vincent and Delachanal, Compt. rend., 104, 1855 (1885).

[18] T. Posternak, Helv. Chim. Acta, 25, 746 (1942).

[19] L. Prunier, Ann. chim. phys., [5] 15, 1 (1878)

[20] H. Muller, J. Chem. Soc., 91, 1766 (1907).

[21] K. H. Bauer and H. Moll, Arch. Pharm., 280, 37 (1942)

quercitol,[22] which choice is unfortunate because the compound is not the optical enantimorph ($[\alpha]_D$ $-74°$) of *d*-quercitol (see above) and because it has no botanical connection with the oak. The configuration is unknown The pentaacetate melts at 124 125°; $[\alpha]_D$ $-26°$.

An inactive cyclohexanepentol, m.p. 233-235°, was synthesized from bioinosose through catalytic hydrogenation in the presence of mineral acid.[23] Its structure follows naturally upon elucidation of the structures of *meso*-inositol and bioinosose (scyllo-*meso*-inosose) (see Fig. 2 for the configurational relationship)

Cyclohexanetetrols are represented by the dextrorotatory betitol, m.p. 221°, isolated in very small amounts from beet sugar process liquors,[24] the synthetic product, dihydroconduritol,[25] m.p. 204°, obtained by hydrogenation of conduritol[26] found in condurango bark, and also the epimeric 1,3-dideoxyinositols[25] (derived from quinic acid by way of the corresponding trihydroxycyclohexanone) which melt at 208° and 151° and show $[\alpha]_D$ 8 3° and $-61 0$, respectively.

Mytilitol, (C'-methylscyllitol) (see Figure 2),[27] m.p. 266 268 , is found in the muscle of *Mytilus edilis*, a mussel [28] The epimeric isomytilitol obtained synthetically through bioinosose is therefore a C-methyl-*meso*-inositol. In both mytilitols the tertiary hydroxyls resist acetylation and both penta- and hexa-acetates can be prepared.

Posternak has also succeeded in synthesizing hydroxymytilitol, m.p. 217 ', and hydroxyisomytilitol, m.p. 223°,[27] from bioinosose by means of the Arndt-Eistert synthesis [29] These are the first known heptahydric homologs of an inositol

Two optically inactive synthetic inositols whose configurations have been elucidated[25] are alloinositol, m.p. 270-275°, and mucoinositol, m.p. 285 290° (decomp.). These were obtained by the permanganate oxidation of conduritol derivatives. It is pointed out that mucoinositol may be identical with Posternak's epiinositol.[30] This is unlikely, however, from the more recent knowledge of the structure of *meso*-inositol.

Epiinositol, m.p. 285° (hexaacetate, m.p. 188°),[30] results from the reduction of inosose (epi-*meso*-inosose).[15]

[22] F B. Power and F Tutin, *J. Chem. Soc.*, **85**, 624 (1904)

[24] T Posternak, *Helv. Chim. Acta*, **24**, 1056 (1941)

[25] E. O. v Lippmann, *Ber.*, **34**, 1161 (1901).

[26] H. O. L Fischer and G. Dangschat, *Naturwissenschaften*, **27**, 756 (1939).

[26] K. Kubler, *Arch. Pharm.*, **246**, 620 (1908).

[27] T. Posternak, *Helv. Chim. Acta*, **27**, 457 (1944).

[28] D. Ackermann, *Ber.*, **54**, 1938 (1921); R. I. Daniel and W. Doran, *Biochem J*, **20**, 076 (1926).

[29] F. Arndt, B. Eistert and W. Ender, *Ber.*, **62**, 44 (1929)

[30] T. Posternak, *Helv. Chim. Acta*, **19**, 1333 (1936).

Isoinositol, m.p. 246 250° (d.), (hexaacetate, m.p. 112°) is formed from *meso*-inositol and scyllitol hexaacetates by the action of hydrogen chloride or hydrogen bromide in acetic acid.[31] This interesting isomerization has not been repeated and could bear verification.

Assuming that isoinositol is homogeneous and not racemic it is evident from the configuration of scyllitol (p. 264, structure IX) that Walden inversion of any one carbon will give *meso* inositol. However, no mention is made of the isolation of *meso*-inositol from the scyllitol inversion product. Should the configuration of any two adjacent carbons in scyllitol be inverted, a racemic mixture of *d*- and *l*-inositol would be formed. For this reason, it is interesting to compare the properties of Müller's isoinositol with the known *d,l*-inositol.

	m p	crystal habit	m p of hexaacetate
d,l inositol	253°	monoclinic	111°
isoinositol	246 250° (d.)	"	112°

In the case of *meso*-inositol, inversion of only one carbon atom (the external ones of the three adjacent *cis* hydroxyls) would be required in order to obtain *d,l*-inositol.

A ψ-inositol described by Müller is not well characterized.

Two inososes (pentahydroxycyclohexanones), both synthetic and both optically inactive, are known. They are obtained from *meso*-inositol. Oxidation with a particular strain of *Acetobacter suboxydans*[5][32] gives scyllo-*meso*-inosose, also termed bioinosose. The other, epi-*meso*-inosose, is obtained in much smaller yield by careful nitric acid oxidation.[30]

Both inososes melt at 200° and both reduce Fehling solution and ammoniacal silver solution instantly in the cold. Scyllo-*meso*-inosose yields a pair of crystalline pentaacetates, m.p. 147° and 211°, and a pair of crystalline pentabenzoates, m.p. 188° and 286°. These are probably dimorphic pairs. Epi-*meso*-inosose, on the other hand, forms only one pentaacetate, m.p. 106 108°, and one pentabenzoate, m.p. 114°.

The acetates of both are aromatized by heating with sodium acetate or pyridine to give 1,2,3,5-tetraacetoxybenzene, whereas the benzoates yield 1-hydroxy-2,3,5-tribenzoxybenzene.[33]

Sodium amalgam reduction in acetic acid produces approximately equal amounts of *meso*-inositol and scyllitol from scyllo-*meso*-inosose, and *meso*-inositol and epiinositol from epi-*meso*-inosose. Catalytic hydrogenation of the latter in neutral solution over platinum oxide forms mainly *meso*-inositol. In acid solution, catalytic hydrogenation of scyllo-*meso*-inosose gives a desoxyinositol.[33] It is evident, consequently, that the two inososes are not identical as was presumed by Kluyver and Boezaardt.

l-Quinic acid, a 1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid,

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[31] H. Müller, *J. Chem. Soc.*, **101**, 2383 (1912).
[32] A. J. Kluyver and A. G. J. Boezaardt, *Rec. trav. chim.*, **58**, 958 (1939).
[33] T. Posternak, *Helv. Chim. Acta*, **24**, 1045 (1941).

m.p. 162°; $[\alpha]_D^{20}$ −44°, is found in cinchona bark, meadow hay, the tops of whortle berries (*Vaccinium myrtillus*, L.), the leaves of the mountain cranberry (*Vaccinium Vitis-idaea*, L.) and combined with caffeic acid[20] as chlorogenic acid in the coffee bean.[14]

d,l-Quinic acid occurs in the heads and leaves of the sugar beet.[35] It may be resolved by means of the quinine or brucine salt.

d-Quinic acid, m.p. 164°; $[\alpha]_D^{20}$ +44°, is obtained from the racemic acid by resolution or by the action of microorganisms, the *l*-form being destroyed.[35]

Shikimic acid, a 1,6-cyclohexene-3,4,5-trihydroxy-1-carboxylic acid, is found in the star anise (*Illicium verum* or *religiosium*).[36]

The relationship between quinic and shikimic acid is illustrated in Fig. 1. The elucidation of the structures is given in the following section.

3. Proofs of Structure and Configuration

A. *meso*-Inositol. The characterization of *meso*-inositol, $C_6H_{12}O_6$, as a cyclohexanehexol was made by Maquenne in 1887[37] on the basis of the presence of six acetylizable hydroxyl groups, the absence of reducing power towards Fehling solution, the indifference to phenylhydrazine, the stability to dilute acids and bases, and particularly, the conversion to triiodophenol and benzene by hydriodic acid, and to the known tetrahydroxybenzoquinone and rhodizonic acid by nitric acid oxidation.

Tetrahydroxybenzoquinone   Rhodizonic acid
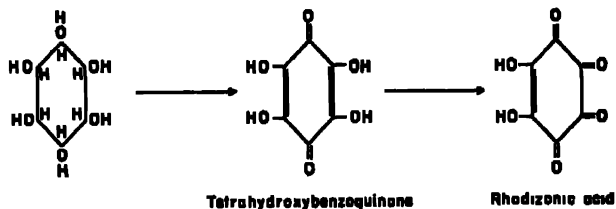
This oxidation reaction is general for the inositols and is the basis for the classical Scherer[38] test for inositol, a red coloration produced by heating the substance with nitric acid followed by the addition of ammonia and calcium chloride.

The total synthesis of inositol by catalytic hydrogenation of hexahydroxybenzene was carried out by Wieland and Wishart[11], *meso*-inositol being the only inositol found. Hexahydroxybenzene, itself, can be prepared by the reaction of carbon monoxide and potassium and subsequent

[14] "Beilsteins Handbuch der organischen Chemie," vol. *10*, p. 535; J. Springer, Berlin (1927).

[35] E. O. von Lippmann, *Ber.*, *34*, 1150 (1901).

[36] J. F. Eijkman, *Rec. trav. chim.*, *4*, 32 (1885); *5*, 299 (1886).

[37] L. Maquenne, *Ann. chim. phys.*, [6] *12*, 1, 112 (1887).

[38] J. Scherer, *Ann.*, *81*, 375 (1852).

acidification with dilute hydrochloric acid. This evidence unequivocally establishes the cyclohexanehexol nature of *meso*-inositol.

By independent means, the configuration of *meso*-inositol was established by Dangschat[39] and by Posternak.[18] Previously, S. and T. Posternak[40] had narrowed the possibilities for *meso*-inositol to

(III)   and   (IV)

by isolating both D,L-talomucic and D,L-*gluco*-saccharic acids from the products obtained by the oxidation of *meso*-inositol with cold alkaline permanganate. Dangschat, making use of the acetonation technic of H. O. L. Fischer (see sections under conduritol, quinic and shikimic acids) acetonated and acetylated *meso*-inositol to a monoisopropylidene tetraacetate. Hydrolysis of the isopropylidene radical followed by lead tetraacetate oxidation and then peracetic oxidation led to the isolation of D,L-*ido*-saccharic acid (see accompanying formulas). Identification of the D,L-*ido* saccharic acid was made by comparison of the diethyl ester and diamide of the acetylated acid with those obtained from L-xylose and D-xylose through the cyanohydrin synthesis. From a consideration of formulas (II) and (IV) it is evident that only (IV) is consistent with the evidence. Hence, the course of the reactions must have been as follows:
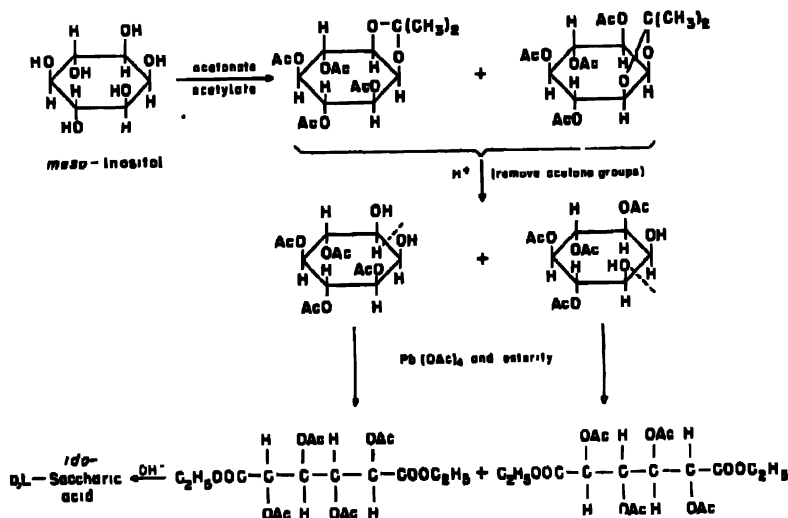
[39] G. Dangschat, *Naturwissenschaften*, **30**, 146 (1942).

[40] S. and T. Posternak, *Helv. Chim. Acta*, **12**, 1170 (1929); T. Posternak, *ibid.*, **18** 1284 (1935).

Posternak, on the other hand, applied the alkaline permanganate oxidation to scyllo-*meso*-inosose (bioinosose) and obtained D,L-*ido*-saccharic (D,L-idaric) acid.[40] This evidence simultaneously establishes the configurations of the inosose, *meso*-inositol and scyllitol. The only configuration compatible with the recovery of D,L-*ido*-saccharic acid from the inosose is:
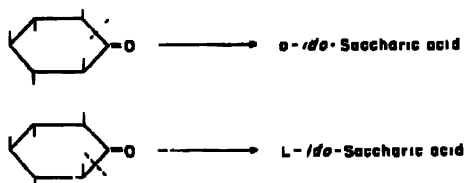
Since *meso*-inositol had previously been limited to configurations (II) and (IV) (p. 264), it must have configuration (IV); scyllitol, an epimer of *meso*-inositol, obtained by reduction of this inosose must have configuration (IX)

B. *d-* **and** *l-***Inositol.** Posternak established the configurations of *d-* and *l-*inositol by isolation of mucic acid and of D-*gluco*-saccharic (glucaric) acid from the products of the cold alkaline permanganate oxidation of *l-*inositol.[41]

The formation of D-*gluco*-saccharic acid requires that the following configuration be present in *l-*inositol:

And since mucic acid was also isolated, there must be another pair of *cis*-hydroxyls. However, because *l-*inositol is optically active, there is only one possible arrangement and that is the projection of the second pair of *cis*-hydroxyls above the plane of the ring. Hence, *d-* and *l-*inositol must be:

l - Inositol   *d* - Inositol

C. *d-***Quercitol.** Homann in his dissertation[42] established conclusively that *d-*quercitol was a pentahydroxy compound by way of the acetate. Prunier[43] meanwhile had aromatized the compound chiefly to benzene by means of hydriodic acid. On the basis of this evidence Kanonnikow (see ref. 44)

[41] T. Posternak, *Helv. Chim. Acta*, *19*, 1007 (1932).

[42] F. W. Homann, Dissertation, Würzburg (1875); *Ann.*, *190*, 282 (1878).

[43] L. Prunier, *Ann. chim. phys.*, [5] *15*, 1 (1878).

proposed a cyclohexanepentol structure. Kiliani and Scheibler,[44] however, believed this structure to be incorrect because when d-quercitol was oxidized with nitric acid mucic acid was obtained. They contended that if Kanonnikow's structure was correct, a trihydroxyadipic (desoxyhexaric) acid should have been formed. In addition to mucic acid a pentaric acid was isolated, identical with that obtained by the oxidation of D-arabinose. However, they indicated that there is insufficient information to attempt to formulate a structure.

In 1926, Karrer[45] reviewed the data on d-quercitol and came to the conclusion that it must have one of the configurations represented in the following two enantiomorphous pairs:

(a)   (b)   (c)   (d)
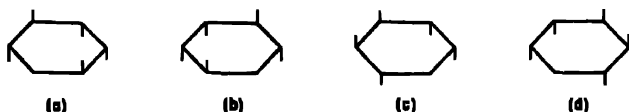
Posternak subjected d-quercitol to cold, alkaline permanganate oxidation[46] and isolated the trihydroxyadipic acid that Kiliani and Scheibler failed to find. This hydroxyadipic acid proved to be identical with the so-called metasaccharonic acid obtained by oxidation of metasaccharin (see below). The configuration of metasaccharin is related to 2-desoxy-D-xylose,[47] obtained from D-xylose through D-xylal but first obtained by Kiliani[48] by the degradation of metasaccharin, and called metasaccharopentose by him.

As 2-desoxy-D-xylose has the configuration $HOCH_2-\overset{\overset{\displaystyle H}{|}}{C}-\overset{\overset{\displaystyle OH}{|}}{C}-CH_2-CHO$
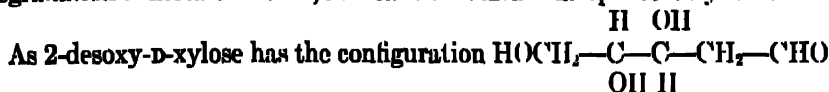
it follows that metasaccharonic acid must likewise contain this configuration. The configuration of the remaining asymmetric carbon is assigned on the basis of the dextro rotation of the phenylhydrazide (Hudson's rule) of the aldonic acid (metasaccharinic acid) obtained from metasaccharin.

Hence, the full configuration of metasaccharonic acid is

$$HOOC-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle HO}{|}}{C}}-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-CH_2-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-COOH$$
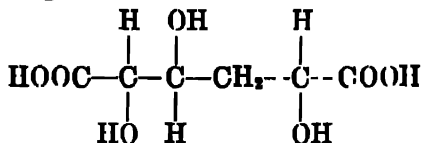
and should be called 3-desoxy-D-galactaric acid; d-quercitol must have configuration (b).

[44] H. Kiliani and C. Scheibler, Ber., 22, 517 (1889)
[45] P. Karrer, Helv. Chim. Acta, 9, 116 (1926).
[46] T. Posternak, Helv. Chim. Acta, 15, 948 (1932)
[47] P. A. Levene and T. Mori, J. Biol. Chem., 83, 809 (1929).
[48] H. Kiliani, Ber., 38, 2069 (1905).

The relationships can best be summed up in the following chart:

d-Quercitol

$$\text{HOOC}-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle HO}{|}}{C}}-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-CH_2-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-COOH$$

3-Desoxy-D-galactaric acid
(Metasaccharonic acid)

$$\text{HOCH}_2-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle HO}{|}}{C}}-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle }{|}}{C}}-CH_2-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-CO$$

Metasaccharin

$$\text{HOCH}_2-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle HO}{|}}{C}}-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-CH_2-CHO$$

2-Desoxy-D-xylose
(Metasaccharopentose)

PhNHNH₂ — PhNHNH$_2$

Phenylhydrazide
$[\alpha]_D + 34.4°$

$$\text{CH}_2-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle HO}{|}}{C}}-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-CH=CH$$

D-Xylal

HNO₃ — HNO$_3$

Ruff degradation
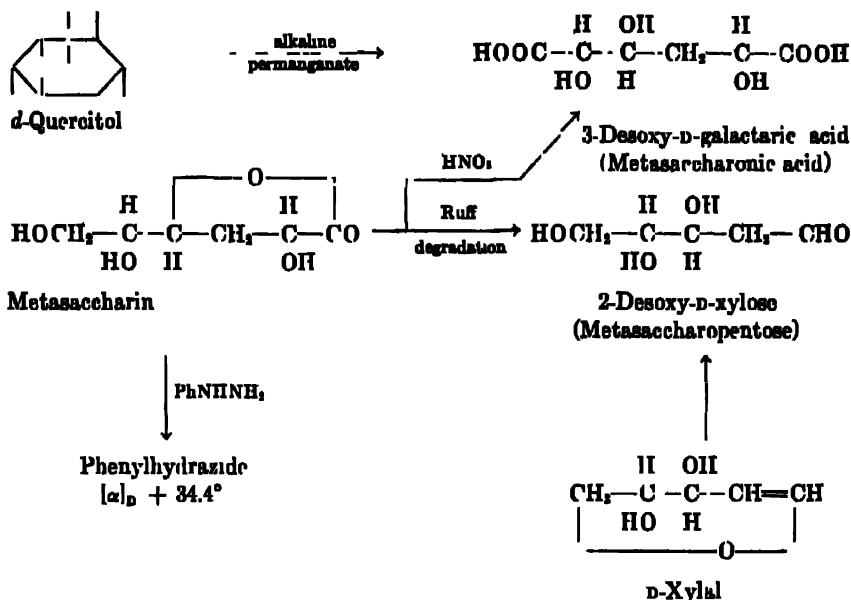
D. Conduritol. The configuration of conduritol, and incidentally those of alloinositol, mucoinositol and dihydroconduritol, was elucidated in 1939 by Fischer and Dangschat[25] who applied the acetonation-oxidation technique previously used so successfully on quinic and shikimic acids. The steps utilized were as follows:

Conduritol

hydrogenation

acetonation

acetylation + KMnO₄

AcO OAc

O O C(CH₃)₂

acetylation, deacetonation oxidation, saponification

deacetonation, saponification

Pb(OAc)₄ oxidation, saponification

Dihydroconduritol

$$\text{HOOC}-\overset{\overset{\displaystyle HO}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle HO}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-COOH$$

Allomucic acid

Alloinositol

$$\text{HOOC}-\overset{\overset{\displaystyle HO}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle HO}{|}}{C}}-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-COOH$$

Mucic acid
(galactaric acid)
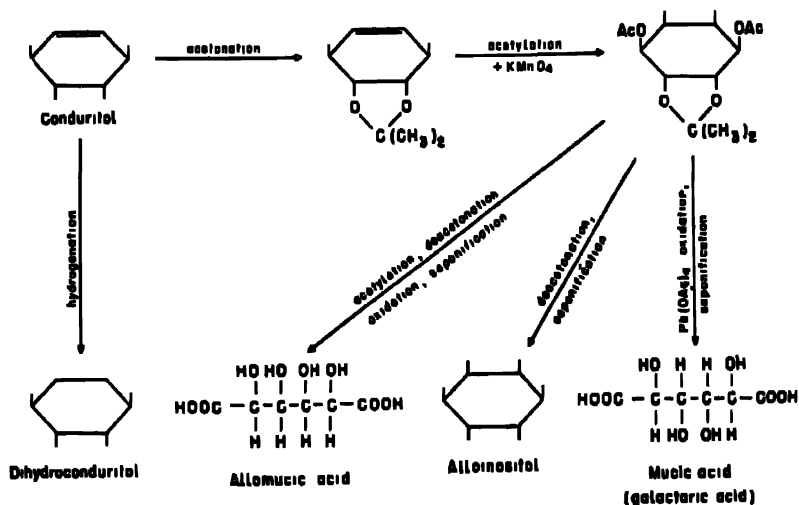
If, however, conduritol was first acetylated then the following results were obtained.

HOOC —C—C—C—C—COOH

Mucic acid
(galactaric acid)

Tetraacetylconduritol

Mucoinositol
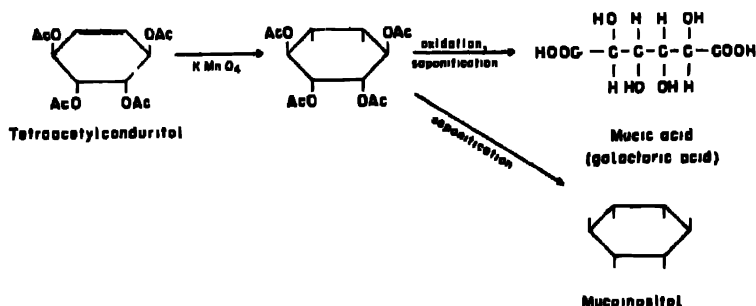
E. Mytilitol. With the establishment of the configuration of scyllo-*meso*-mosose (see p. 273), Posternak was able to proceed with the configuration of mytilitol[7] and a number of synthetic products, isomytilitol, hydroxymytilitol and hydroxyisomytilitol, through the following series of reactions

Scyllo-*meso*-inosose

Mytilitol

Isomytilitol

Hydroxymytilitol

Hydroxyisomytilitol
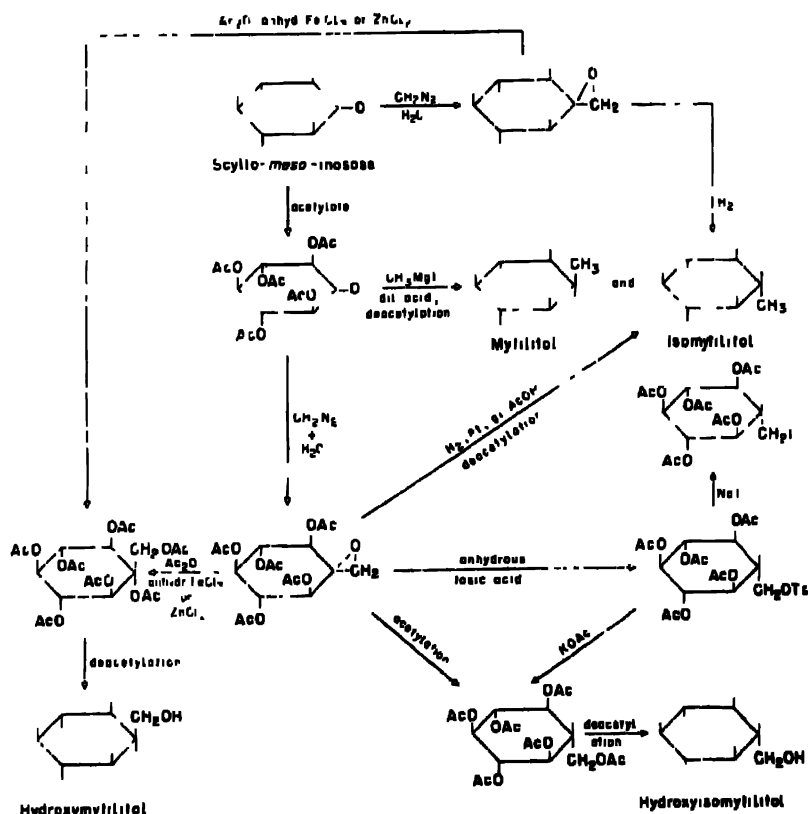
In the Grignard reaction, mytilitol and isomytilitol are formed from the

pentaacetylinosose. The configuration with three adjacent *cis* hydroxyl groups was assigned to isomytilitol and the other to mytilitol by analogy to the periodic acid oxidation of scyllitol and *meso*-inositol. Scyllitol has a completely *trans* configuration and is oxidized more slowly than *meso*-inositol. Similarly mytilitol is attacked less rapidly than isomytilitol.

Since hydrogenation of both the epoxide derivative of scyllo-*meso*-inosose and of its pentaacetate (obtained through the Arndt-Eistert synthesis) produces isomytilitol, the configuration of the tertiary carbon atom is established inasmuch as the oxygen remains with the tertiary carbon during scission of the epoxide group. Scission of the epoxide ring in the pentaacetyl derivative with either acetic acid or *p*-toluenesulfonic acid, likewise, does not involve the tertiary carbon atom, for the ready replacement of the tosyloxy group by iodine indicates a primary ester linkage. Therefore, the hydroxy derivative appears to be configurationally related to isomytilitol.
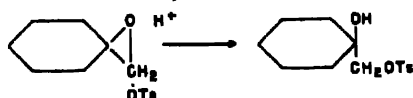
On the other hand, acetylation with acetic anhydride in the presence of anhydrous ferric chloride or zinc chloride apparently involves an opening of the ethylene oxide ring at the tertiary carbon with consequent inversion, for the hydroxy derivative ultimately obtained is not hydroxyisomytilitol. Hence, it would seem to be the epimer configurationally related to mytilitol.

F. Quinic Acid. The burden of the proof of configuration of quinic acid rests on a series of reactions involving the acetone derivative and its lactone (quinide) [49] The following scheme illustrates the reactions involved.
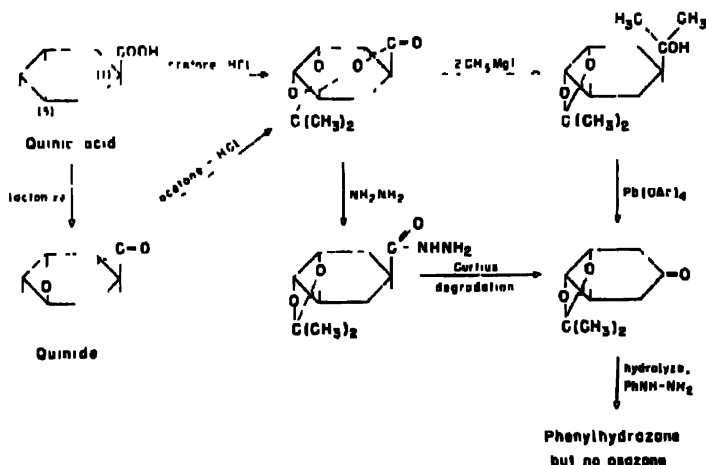
[49] H. O. L. Fischer. Ber., *54*, 775 (1921); H. O. L. Fischer and G. Dangschat, *ibid.*, *65*, 1009 (1932).

It had been previously established that quinic acid readily forms a lactone called quinide. Quinide was shown to have a γ-lactone structure by conversion of the trimethyl ether to 3-hydroxy-4-methoxybenzoic acid (isovanillic acid). At the same time, this reaction established that the hydroxyl at carbon 3 and the carboxyl must be on the same side of the ring. Since the hydroxyl derivative obtained through the Grignard reaction consumes one mole of lead tetraacetate and from the results of the Curtius degradation, it follows that carbon 1 must have both a carboxyl and hydroxyl attached. Furthermore, since the resultant ketone cannot form an osazone, carbons 2 and 6 must be free of hydroxyl groups. By elimination, therefore, the remaining two hydroxyls must be at carbons 4 and 5. Finally, these must be *cis* in order to form an acetone derivative and be *trans* to the hydroxyl at carbon 3 because quinic acid is optically active.

G. Shikimic Acid. The configuration of shikimic acid was established by H. O. L. Fischer and G. Dangschat[50] through the following series of reactions.

2-Desoxygluconic acid
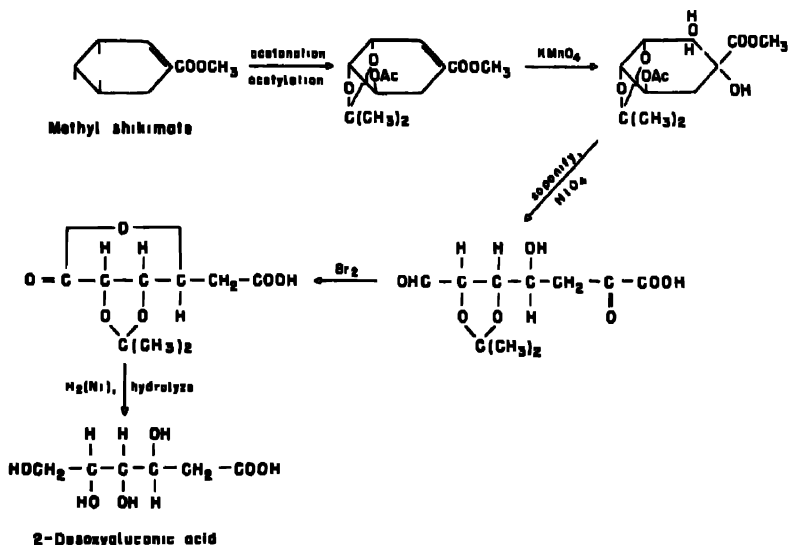
These steps leave no question regarding the configuration of shikimic acid and the position of the double bond.

The structural similarities among quinic, shikimic and gallic acids are striking and their possible relationship in the plant are discussed by Fischer and Dangschat.

[50] H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, **17**, 1200 (1934); **20**, 705 (1937).
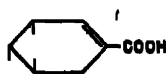
d - Quinic acid   Shikimic acid   Gallic acid
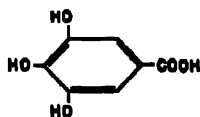
4. Reactions

The reactions of the cyclitols are those of the polyhydric alcohols, but the presence of the ring structure exerts an important modifying influence.

A. Behavior with oxidizing agents.

a. NITRIC ACID

The cyclitols are resistant to oxidation with dilute nitric acid but with concentrated acid, depending on the conditions, a variety of products may be obtained ranging from carbon dioxide to cyclic ketones. Reference will be made here, as well as in the subsequent reactions, to those instances in which the ring has remained intact or in which it has been opened and compounds retaining all the original carbons have been isolated.

The Scherer test[38] for meso-inositol is dependent on the formation of rho dizonic acid, whose calcium salt has a red color. This test is given by all the known inositols and will probably be given by those not as yet synthesized. It is not satisfactory when applied to the methyl ethers.[51] Salkowski[52] has modified the Scherer test so that as little as 0.1 mg. of inositol can be detected. The test is carried out as follows: A little inositol is dissolved in 1–2 drops of nitric acid (sp. g. 1.2), a drop each of 10% CaCl· and 1–2% H_2PtCl_6 solutions are added, and the mixture is cautiously concentrated on a porcelain crucible cover. A rose to brick-red color appears.

Bartow and Hoglan, as well as Preisler and Berger,[53] give detailed directions for the preparation of rhodizonic acid in quantity from meso-inositol. The reaction apparently goes through the following steps:

Tetrahydroxybenzoquinone   Rhodizonic acid
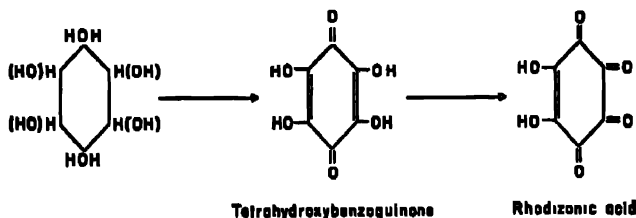
Other higher oxidation products are formed at the same time.

[51] P. Dekker, Kautschuk, 13, 110 (1937).
[52] E. Salkowski, Z. physiol. Chem , 69, 475 (1910).
[53] E. Bartow and F. A. Hoglan, J. Am Chem Soc , 62, 2397 (1940); P. W. Preisler and L. Berger, ibid , 64, 67 (1942)

Posternak[20] oxidized *meso*-inositol to epi-*meso*-inosose, a pentahydroxy-cyclohexanone (see above). The yield was poor.

d-Quercitol has been oxidized to mucic (galactaric) acid.[44]

b. ALKALINE PERMANGANATE

This oxidation, as employed by Posternak, was very useful in elucidating the configuration of the inositols (see above). From *meso*-inositol S. and T. Posternak obtained D,L-*talo*-saccharic (talaric) and D,L-*gluco*-saccharic (glucaric) acids. From scyllo-*meso*-inosose, T. Posternak obtained D,L-*ido*-saccharic (idaric) acid. In these instances, the ring was opened to form dibasic acids. *d*-Quercitol was oxidized by Posternak[46] to metasaccharonic acid. In all these oxidations, it was necessary to maintain low temperatures.

d-Quercitol was oxidized to benzoquinone by Prunier[54] with manganese dioxide in sulfuric acid.

l-Quinic acid was oxidized to benzoquinone by Wöhler.[55] Derivatives of conduritol and shikimic acid were hydroxylated at the double bond by Fischer and Dangschat.[25] [50]

c. HYPOBROMITE AND BROMINE

d-Quercitol was oxidized by Kiliani and Schäfer who used bromine on an aqueous solution of the cyclitol. They obtained a cyclohexanetrioldione characterized as the bisphenylhydrazone, m.p. 180° (d.).[56]

l-Quercitol was oxidized by Power and Tutin[57] to a cyclohexanetrioldione using sodium hypobromite. They characterized the compound as the bis-phenylhydrazone, m.p. 209° (d.).

d. TETRAVALENT LEAD

Lead tetraacetate has been employed for the oxidation of the muco- and allo-inositol derivatives obtained by hydroxylation of the corresponding conduritol compounds.[25] Ultimately, there were isolated and identified the dibasic acids corresponding to the dialdehydes obtained by breaking the cyclitol ring.

Lead dioxide was used by Hesse[55] to oxidize *l*-quinic acid. He obtained hydroquinone. Evidently decarboxylation resulted as above.[55]

e. BACTERIAL OXIDATION

Scyllo-*meso*-inosose ("bioinosose"), a pentahydroxycyclohexanone, was obtained by the action of a special strain of *Acetobacter suboxydans*[18] [32] on *meso*-inositol.

[51] L. Prunier, *Ann. chim. phys.*, [5] *15*, 54 (1878).
[55] F. Wöhler, *Ann.*, *51*, 148 (1844).
[56] H. Kiliani and J. Schäfer, *Ber.*, *29*, 1765-6 (1896).
[57] F. B. Power and F. Tutin, *J. Chem. Soc.*, *85*, 628 (1904).
[58] O. Hesse, *Ann.*, *114*, 296 (1860).

Dunning reports the isolation of a diketo derivative of *meso*-inositol[54] also formed by a strain of *A. suboxydans*, but the physical constants of the compound, the acetate and the bisphenylhydrazone are not given. At present, the identification is questionable.

B. Reaction with Halogen Acids. The reactions of the cyclitols with halogen acids may be divided into two groups, halohydrin formation and aromatization.

a. HALOHYDRIN FORMATION

There appears to be only one example of halohydrins obtained by direct action of halogen acids on the cyclitols; *d*-quercitol was heated at 100° with a solution of HCl (saturated at 10°), and a very small amount of substance, m.p. 198–200°, was obtained which had an analysis corresponding to a monochlorohydrin,[54] and also one, m.p. 155°, that appeared to be a trichlorohydrin, $C_6H_7Cl_3(OH)_3$.

A number of such derivatives have been obtained from cyclohexanehexol esters through the action of HCl or HBr on the ester[3] or by reacting an acyl halide with a cyclitol.[60,b] In the latter case, esterification, more or less complete, probably occurs initially; this is followed by the action of the liberated halogen acid. The known halohydrins and esters are listed in Table III.

Although the configurations of these substances are unknown, there is some evidence which makes it possible to group those of like configuration. It must be kept in mind that these configurations may differ from those of the original cyclitols in view of Muller's isomerization of *meso*-inositol and scyllitol hexaacetates[31] and the fact that pinitol (the monomethyl ether of *d*-inositol) apparently gives the same dibromohydrin derivatives as *meso*-inositol (see Table III).

The dichlorohydrin tetraacetate, m.p. 118°, is an ester of the dichlorohydrin, m.p. 221°, for the latter, when acetylated, gives a product with a melting point of 118°; the same dichlorohydrin and tetraacetate can be obtained from the triacetate.

The dibromohydrin, m.p. 216°, the dibromohydrin diacetate, m.p. 214°, and the triacetate, m.p. 121°, when acetylated, produce the dibromohydrin tetraacetate having a melting point of 130°. Muller's[31] two dibromohydrin tetraacetates are probably identical with those obtained by Griffin and Nelson[60a] (as pointed out by the latter authors) because the crystal habits are very similar and because repetition of Muller's procedure by these workers yielded only the derivatives melting at 225 and 130°.

It also seems likely that the dichlorohydrin tetraacetates are configurationally identical with the corresponding dibromohydrin tetraacetates, for

[54] J. W. Dunning, *Iowa State Coll. J. Sci.*, **14**, 24 (1939).

[60a] E. G. Griffin and J. M. Nelson. *J. Am. Chem. Soc.*, **37**, 1552 (1915).

[60b] L. Maquenne, *Compt. rend.*, **104**, 1720 (1887).

these halohydrins acetates appear to be isomorphous; the parent halohydrins also are isomorphous.

<div align="center">

TABLE III

Halohydrins of Inositols

</div>

Derivative	M.P. (°C.)	Ref.
A. *meso*-Inositol Derivatives		
Monochlorohydrin pentaacetate	109–110	31
" "	118	31
" "	240–247 (250)	31, 60a
Monochlorohydrin triacetate	145	31
Monochlorohydrin	180–185	31
Chlorohydrin benzoate	—	60b
Dichlorohydrin tetraacetate (α)	186	60a
" " (β)	118	60a
Dichlorohydrin triacetate	-	60a
Dichlorohydrin	221	60a
Tribromohydrin triacetate	180	60a
Dibromohydrin tetraacetate (α)	225	60a
" " (β)	130	60a
" "	235	31
" "	140	31
Dibromohydrin triacetate	124	60a
Dibromohydrin diacetate	211 (d)	60a
Dibromohydrin	216	60a
"	210 (d)	31
Monobromohydrin pentaacetate	240	31, 60a
Monobromohydrin	170–175	31
B. Scyllitol Derivatives		
Monochlorohydrin	200 (d)	31
Dibromohydrin tetraacetate	235	31
Monobromohydrin pentaacetate	240	31, 60a
Monobromohydrin	170-175	31
C. Pinitol Derivatives		
Dibromohydrin tetraacetate (α)	225	60a
" " (β)	130	60a
Dibromohydrin	216	60a

Two bromohydrins have been synthesized by Kubler by other means.[25] Conduritol, treated with bromine water at 50°, yielded a dibromocyclohexanetetrol, m.p. 176°, and a monobromocyclohexanepentol, m.p. 175°. The latter agrees in melting point with Müller's monobromohydrin, m.p. 170-5°, obtained from scyllitol and from *meso*-inositol. It is possible that

the two may be identical[51] although the melting point of Müller's compound would indicate that it had not been completely purified.

b. Aromatization

The halogen acids acting directly on the cyclitols generally produce substances of a benzenoid nature. This behavior is especially characteristic of hydriodic acid, although instances where hydrochloric and hydrobromic acids have acted similarly are reported. Thus, Maquenne reported the isolation of 2,4,6-triiodophenol, phenol and other aromatic substances[52] by the action of fuming HI at 150 170° on d- and meso-inositol. Lautemann[53] reported benzoic acid from l-quinic acid using HI. Prunier[54] obtained benzene, phenol, hydroquinone and benzoquinone from d-quercitol. Oswald[64] obtained benzoic acid by heating an aqueous solution of shikimic acid with PI₃.

Hydrochloric acid has been used for the aromatization of shikimic acid to p-hydroxybenzoic acid[65], and of l-quinic acid to hydroquinone and p-hydroxybenzoic acid.[66] Conduritol was transformed in part to catechol by 12.5 to 25% HCl[57] Fuming hydrobromic acid converted l-quinic acid to hydroquinone, 3,4-dihydroxybenzoic and benzoic acids.[67] Müller[51] reported the isolation of a small amount of bromobenzene as a result of the treatment of inositol hexaacetate with a glacial acetic acid solution of HBr.

Such aromatization is not peculiar to the halogen acids, for sulfuric acid or alkali at high temperatures or even heat alone will cause the dehydration of certain of the cyclitols to aromatics.[68]

The pentaacetate and pentabenzoate of scyllo-meso- and epi-meso-inosose when heated with pyridine or sodium acetate form 1,2,3,5-tetraacetoxybenzene and 1-hydroxy-2,3,5-tribenzoxybenzene, respectively.

C. Esterification. Esterification of the cyclitols may be carried out using the free acid, acid anhydride or acid chloride with or without the presence of catalysts in the usual manner (see Chapter IV). However, partial esters have in some instances been recovered from reactions in which full esterifica-

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[51] Since d- and l-inositol rather than meso-inositol contain the same configuration as conduritol, a Walden inversion presumably occurs in the preparation of Müller's derivative and bromine enters one of the two neighboring positions.

The addition of HOBr to conduritol might yield four enantiomorphous pairs of monobromocyclohexanepentols. Kubler apparently obtained only one d,l pair, the same one synthesized by Müller.

[52] L. Maquenne, Compt. rend., 104, 297 (1887); 109, 813 (1889).
[53] E. Lautemann, Ann., 125, 9 (1863).
[54] F. Oswald, Arch. Pharm., 229, 100 (1891)
[65] J. F. Eijkman, Ber., 24, 1286 (1891).
[66] O. Hesse, Ann., 200, 238 (1880).
[67] R. Fittig and W. F. Hillebrand, Ann., 193, 197 (1878).
[68] "Beilsteins Handbuch der organischen Chemie," vol. 6, p 1186; J Springer, Berlin (1923); vol. 10, p. 458, 536 (1927).

tion was desired. The distribution of the acyl groups in these compounds is not known.

An interesting case of partial esterification is the acylation of *l*-quinic acid. If the acid and acetic anhydride are refluxed briefly, one obtains tri-acetylquinide (the lactone of quinic acid). If zinc chloride is present tetra-acetylquinic acid is formed.[69] However, if quinic acid and benzoyl chloride are heated at 130 to 140° the main product is tetrabenzoylquinic acid. If the reaction is carried out in the presence of pyridine, tribenzoylquinide is the chief product.[70]

Some phosphate esters of *meso*-inositol have been prepared[71] in order to arrive at a better understanding of the structure of phytic acid; this natural hexaphosphate appears to be of considerable importance biochemically to many plants (see under occurrence of *meso*-inositol).

Maquenne prepared the hexanitrate and found that it detonates with shock.[72]

D. Alkylidene Formation. Acetals and ketals of the cyclitols have not been prepared to any considerable extent. It might be expected that this type of compound could be useful in elucidating configurations as was shown by Fischer and Dangschat in their work on shikimic acid, quinic acid, and conduritol.[2b, 49, 50]

The configuration of the naturally occurring methyl ethers as well as those of the various halohydrins remain to be determined, and the problem undoubtedly could be solved by applying the technique of Fischer or of Posternak.

Some other examples of alkylidene derivatives are 3-acetyl-4,5-methyl-enequinic amide, m.p 149°, and 4,5-methyleneshikimic acid, m p. 138°, |α|ᴅ 88 7° (H₂O).[73]

E. Metallic Complexes. The cyclitols can form complexes with metals similar to those of the acyclic polyhydric alcohols.

The formation of an insoluble reaction product with basic lead acetate is a means of removing *meso*-inositol almost quantitatively from solution.[74]

F. Miscellaneous Reactions. If *meso*-inositol is treated with thionyl chloride in the presence of pyridine[75] there are formed a monochloro-cyclopentitol, decomp. 248°, a tetrachlorocyclohexanediol, m.p 186°-187°, and some polychlorobenzenes and polychlorophenols.

There is only one instance reported in the literature of the preparation of

[69] E. Erwig and W Koenigs, *Ber*, **22**, 1458 (1889)

[70] P. Echtermeier, *Arch Pharm.*, **244**, 46 (1906)

[71] S. Posternak, *Helv Chim Acta*, **4**, 150 (1921); R. J. Anderson, *J. Biol. Chem*, **13**, 97 (1912)

[72] L. Maquenne, *Compl rend*, **104**, 1719 (1887)

[73] G Dangschat and H. O. L Fischer, *Naturwissenschaften.* **26**, 562 (1938)

[74] G Meillère and P Fleury, *J. pharm. chim.*, [7] **1**, 384 (1911).

[75] R Majima and H. Simanuki, *Proc Imp. Acad. (Tokyo)*, **2**, 544 (1926)

an external ether. This was obtained from d-quercitol by Prunier[44] who
heated the cyclitol at 235–250°. A compound, m.p. 228–230° and having the
composition $C_{12}H_{22}O_{9}$, sublimed. Evidently, a dicyclitol ether was formed.
The residual sirup contained a small amount of substance (quercitan) which
may be an internal ether.

An example of an epoxy type of anhydride was obtained by Posternak
employing the Arndt-Eistert synthesis while elucidating the structure and
configuration of mytilitol (see page 276).

A probable epoxy compound was obtained by Eijkman[65] who treated
shikimic acid with bromine and obtained a dibromo compound, m.p. 188°,
$[\alpha]_D$ −58° (H_2O). On treatment with 0.5 mole of moist silver oxide, it
lost one mole of HBr forming a monobromohydrin, m.p. 235°, $[\alpha]_D$ +22°
(H_2O). The monobromohydrin on treatment with aqueous barium hydroxide
gave a carboxy cyclopentitol. The reaction, therefore, probably proceeded
as follows:

Shikimic acid   Dihydroxyshikimic acid
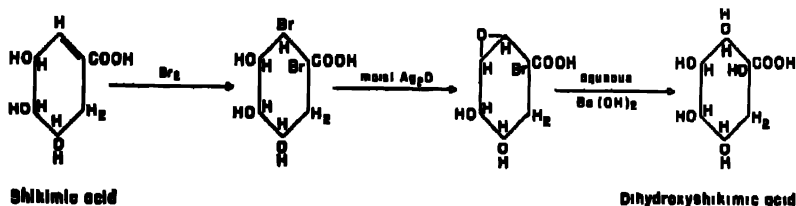
This pentahydroxy acid begins to melt at 156° with the splitting out of
water (probably lactonization).

Griffin and Nelson[60a] found that meso-inositol was resistant to alkylation
by most of the usual etherifying agents except dimethyl and diethyl sul-
fate. They obtained a monomethyl ether, m.p 204°, its pentaacetate, m.p.
141°, also the tetraacetate, m.p. 223°, of a dimethyl ether which was ob-
tained as a sirup after saponification of the acetate. They also obtained a
sirup which has an analysis corresponding to a triacetyl dimethyl ether.
With diethyl sulfate a monoethyl derivative was isolated as the pentaace-
tate, m.p. 128°. They also isolated a tetraacetyl diethyl ether, m.p. 212°,
which was saponified to the free diethylcyclitol, m.p. 212°.

An aryl ether was synthesized by Milhorat and Bartels[76] who reacted
benzene hexachloride and α-tocopherol in absolute alcoholic KOH under
nitrogen with simultaneous etherification and hydrolysis of the benzene
hexachloride. This is noteworthy because it is the first successful hydrolysis
of benzene hexachloride. However, the cyclitol portion may not necessarily
have the meso-inositol configuration.

Because of the considerable stability of the inositols to hydriodic acid of
constant boiling point, it is possible to obtain quantitative recoveries of the
inositols from their naturally occurring methyl ethers.

[76] A. T. Milhorat and W. E. Bartels, Science, 101, 93 (1945).

5. Biochemistry

The inositol compounds were early recognized as of great importance because of their wide distribution in nature. Hence, it was natural to attempt to synthesize these substances.

While the total synthesis of *meso*-inositol has been carried out starting with potassium and carbon monoxide[11] no synthesis *in vitro* has approached that accomplished *in vivo*. The most direct method, obviously, would be the cyclization of an aldohexose by an internal aldol condensation. Micheel and associates[77] made a number of unsuccessful attempts in this direction. Micheel also reacted the 1,6-diiodohydrin of dimethylenemannitol with "molecular" silver in toluene or xylene at 165-170° and obtained two dimethylene derivatives which on hydrolysis yielded the free polyols. One, melting at 229°, $[\alpha]_D^{19}$ + 31.6 (H_2O), was believed to be tetrahydroxymannocyclitol and the other, melting at 148°, $[\alpha]_D^{19}$ − 17.6° ($CHCl_3$) was believed to be 1,6-didesoxymannitol.[78]

The structure of Micheel's second compound, as opposed to the views of Hamamura, was confirmed by reduction of the 1,6-diiodo-2,3,4,5-dibenzylidene-D-mannitol with Raney nickel and subsequent removal of the benzylidene groups.[79]

It appears, therefore, that "molecular" silver obtained by reducing silver chloride with formaldehyde contains sufficient adsorbed hydrogen to reduce the iodo derivative to the desoxy compound.

Since Eastcott's work on yeast growth factors[80] wherein *meso*-inositol was shown to be the so-called Bios I, further research has established this inositol as a vitamin not only for the lower organisms but also for the higher animals. It is a constituent of the vitamin B complex.[81] Williams believes that in a 2500 calory diet approximately 981 mg. daily is required.[82]

meso-Inositol has a favorable effect on certain types of fatty livers,[83] alone or in conjunction with other members of the B group of vitamins.

[77] F. Micheel, H. Ruhkopf and F Suckfull, *Ber.*, **68**, 1523 (1935).

[78] F. Micheel, *Ann.*, *496*, 77 (1932), cf., Y. Hamamura, *Proc. Imp Acad (Tokyo)*, *10*, 459 (1934); *Chem. Abst.*, *29*, 1070[1] (1935).

[79] W. T. Haskins, R. M Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 1419 (1943).

[80] E. V. Eastcott, *J. Phys Chem*, *32*, 1094 (1928).

[81] H. R. Rosenberg, "Chemistry and Physiology of the Vitamins"; Interscience Publishers, New York (1942).

[82] R. J. Williams, *J. Am. Med Assoc.*, *119*, 1 (1942).

[83] J. C. Abels, C. W. Kupel, G T Pack and C. P. Rhoads, *Proc. Soc. Exptl. Biol. Med.*, *54*, 157 (1943); J. C. Forbes, *ibid.*, *54*, 89 (1943); G. Gavin, J. M. Patterson and E. W. McHenry, *J. Biol Chem*, *148*, 275 (1943); M. L. MacFarland and E. W. McHenry, *ibid.*, *148*, 275 (1943); M. L. MacFarland and E. W. McHenry, *ibid.*, *159*, 605 (1945).

meso-Inositol corrects the condition in the rat known as spectacle eye.[54] It is also effective against mouse alopecia.

Woolley has compared the activities of compounds related to *meso*-inositol for their curative effect on dietary alopecia of the mouse.[55] He found that phytin, mytilitol, soybean cephalin and *meso*-inositol hexaacetate were active while *d*- and *l*-inositol, *d*-quercitol, quebrachitol and pinitol were not. *meso*-Inositol is found in human hair along with other B complex vitamins, and Novak and Bergeim have indicated that there may be a relationship between the inositol content and certain types of human baldness.[56] They found the inositol to be low in these cases.

In the case of certain malignant mouse tumors *meso*-inositol was found to bring about regression of the tumor.[57] Laszlo and Leuchtenberger have found that the addition of *meso*-inositol to the diet inhibited tumor growth in mice.[58] Milhorat and Bartels synthesized an α-tocopherol ether of an inositol-like substance and found it to be many times more effective in cases of muscular dystrophy than wheat germ oil or the ethylene dichloride extract of wheat germ. It was also much more effective than oral administration of *meso*-inositol and α-tocopherol.[75]

The stimulation of growth of certain types of bacteria has led to the development of a microbiological assay method for *meso*-inositol in natural products.[59] This growth stimulation appears to be more specific than the cure of alopecia in mice. Mytilitol and phytin, both active against alopecia, had 10% and 1%, respectively, of the stimulating effect of *meso*-inositol on yeast growth. Other related compounds ranged from 5% for *meso*-inositol monophosphate to 1% for *d*- and *l*-inositol, pinitol, quebrachitol and *meso*-inositol hexaacetate.[54]

Extremely interesting is the isolation of a guanidine derivative from streptomycin that appears to contain an aminated cyclitol[90] of the following structure.
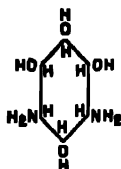
[54] P. L. Pavcek and H. M. Braun, *Science*, *93*, 502 (1941).

[55] D. W. Woolley, *J. Biol. Chem.*, *140*, 461 (1941).

[56] L. J. Novak and O. Bergeim, *J. Biol. Chem.*, *155*, 283 (1944)

[57] M. L. Hesselbach and D Burk, *Record Chem. Progress*, *5*, 37 (1944).

[58] D. Laszlo and C. Leuchtenberger, *Science*, *97*, 515 (1943).

[59] V. Jurist and J. R. Foy, *J. Bact.*, *47*, 434 (1944).

[90] R. L. Peck, C. E. Hoffhine, Jr , E. W Peel, R R Graber, F. W. Holly, R Mozingo and K. Folkers, *J. Am. Chem Soc*, *68*, 776 (1946); H. E. Clark, R K Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *Science*, *103*, 540(1946).

ACIDS AND OXIDATION PRODUCTS OF CARBOHYDRATES[1,2]

Aldonic acids, saccharic acids, ascorbic acids and analogs and uronic acids are the most important classes of acidic carbohydrates. Some of these acids have achieved commercial importance, particularly ascorbic acid and gluconic acid, and the others have interesting potentialities. Slightly oxidized polysaccharides, particularly starch and cellulose (discussed under these substances in later chapters), provide commercially valuable modifications of these materials, although the nature of the oxidation has not received much scientific investigation. Naturally occurring acids include ascorbic acid, tartaric acid and the uronic acids. Other acids are produced as a result of the action of microorganisms on carbohydrates and are found in natural products.

The characteristic oxidizable groupings in the carbohydrate series are:

$$\text{CHO} \quad \text{or} \quad \overset{|}{\underset{|}{\text{HOCH}}}\ \overset{--}{\text{O}}, \quad \overset{|}{\underset{|}{\text{HCOH}}}, \quad \overset{|}{\underset{|}{\text{CH}_2\text{OH}}} \quad \text{and} \quad --\overset{|}{\underset{|}{\text{C}}}--\overset{|}{\underset{|}{\text{C}}}$$

Some typical examples of oxidation reactions and products are given below.

$$
\begin{array}{ccccc}
\text{CHO} & & \text{COOH} & & \text{COOH} \\
| & & | & & | \\
(\text{CHOH})_n & \rightarrow & (\text{CHOH})_n & \rightarrow & (\text{CHOH})_n \\
| & & | & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{COOH} \\
\text{Aldoses} & & \text{Aldonic acids} & & \text{Saccharic acids} \\
& & & & \text{(Aric acids)} \\
\text{(I)} & & \text{(II)} & & \text{(III)}
\end{array}
$$

$$
\begin{array}{ccc}
\text{CHO} & & \text{COOH} \\
| & & | \\
(\text{CHOH})_n & \rightarrow & (\text{CHOH})_n \\
| & & | \\
\text{COOH} & & \text{COOH} \\
\text{Uronic acids} & & \text{Saccharic acids} \\
& & \text{(Aric acids)} \\
\text{(IV)} & & \text{(V)}
\end{array}
$$

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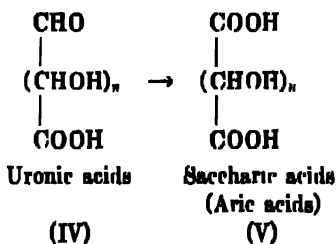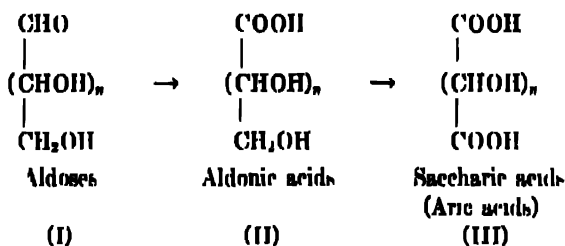
[1] Prepared by John W. Green.

[2] Some general references to oxidation reactions are: *Trans. Faraday Soc.*, **42**, 99–308 (1946); W. A. Waters, *Ann. Repts. on Progress Chem.* (*Chem. Soc. London*), **42**, 130 (1945); L. J. Heidt, E. K. Gladding and C. B. Purves, *Paper Trade J.*, **121**, 81 (1945).

$$
\begin{array}{cccc}
\text{CH}_2\text{OH} & \text{CHO} & \text{CHO} & \text{COOH} \\
| & | & | & | \\
(\text{CHOH})_n \rightarrow & (\text{CHOH})_n \rightarrow & \text{CO} & \rightarrow \text{CO} \\
| & | & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & (\text{CHOH})_{n-1} & (\text{CHOH})_{n-1} \\
 & & | & | \\
 & & \text{CH}_2\text{OH} & \text{CH}_2\text{OH}
\end{array}
$$

| Glykitols (Polyols) (VI) | Aldoses (VII) | Osones (VIII) | 2-Ketoaldonic acids (IX) |

$$
\begin{array}{ccc}
\text{HCOCH}_3 & \text{HCOCH}_3 & \text{HCOCH}_3 \\
| & | & | \\
\text{CHOH} & \text{CHOH} & \text{CHO} \\
| \quad \text{O} \leftarrow & | \quad \text{O} \rightarrow & | \quad \text{O} \\
\text{CHOH} & \text{CHOH} & \text{CHO} \\
| & | & | \\
.\text{HC} & \text{HC} & \text{HC} \\
| & | & | \\
\text{COOH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH}
\end{array}
$$

| Uronides (X) | Glycosides (XI) | Dialdehydes (XII) |
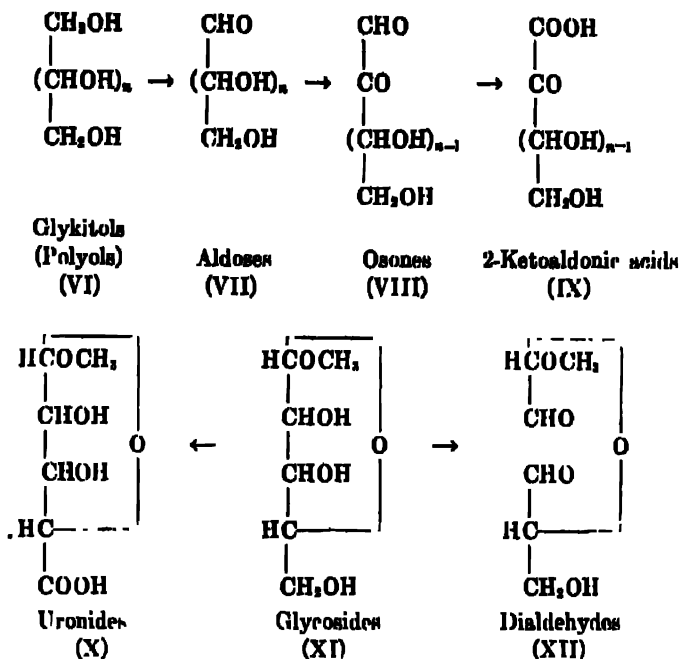
The most commonly employed oxidative agents are halogens and oxyhalogen acids, nitric acid and hydrogen peroxide. The general field of oxidants has not been explored systematically, and the oxidative mechanisms have received but little study.[a] Relatively few oxidation reactions follow a single course or give high yields of single products. Probably the bromine or hypoiodite oxidation of aldoses to aldonic acids, the nitric acid oxidation of galactose to mucic acid, and the periodic acid oxidation of glycol-containing compounds represent reactions with highest yields. Ordinarily, the primary oxidation product may be further oxidized ("over-oxidation"), or several groups may be attacked simultaneously.

The aldehyde (or hemiacetal) group is the most easily oxidized common group found in carbohydrates. Bromine and hypoiodite convert it readily to the carboxyl (or lactone) group. Most other agents simultaneously attack other points of the molecule, although nitric acid (or nitrous acid) may have some value for this type of reaction.

Primary alcoholic groups (--CH₂OH) may be converted to aldehyde and carboxyl groups by agents such as nitric acid, hypoiodites and platinic oxide, but the yields are usually low.

Secondary alcoholic groups [- -CH(OH)—], particularly those in the 2

[a] For such studies, see later sections on hydrogen peroxide, bromine and periodic acid oxidation.

and 5 positions of hexose derivatives, can be converted to keto groups, especially if other oxidizable groups in the molecule are blocked. Permanganate and oxyhalogen salts, the latter in the presence of catalysts, have been used for the purpose, but again the yields are poor.

Most oxidative reagents will bring about cleavage of carbon-carbon bonds under sufficiently drastic conditions. Permanganates, chromates and cerates may cause quantitative decomposition into carbon dioxide, formic acid and formaldehyde. On the other hand, hydrogen peroxide (with ferric salts as catalyst) and oxygen in alkaline solution produce cleavage between carbons 1 and 2 of aldonic acids and sugars, respectively; the reactions are sufficiently specific to be of value for preparatory purposes. The cleavage of vicinal glycol groups (CHOH   CHOH—) by periodic acid or lead tetraacetate, usually to dialdehydes, is extremely specific and important.

. The remainder of this chapter will be devoted, first, to a discussion of the preparation and chemistry of carbohydrate acids and oxidation products and, finally, to the effect of specific oxidants.

1. Preparation and Reactions

A. Aldonic Acids. The aldonic acids are the initial oxidation products produced from aldoses by most oxidants and are usually isolated as the metallic salts or the lactones. As a result of the ease with which the crystalline lactones, salts, amides, hydrazides and other derivatives can be formed,

$$R—(CHOH)_n \quad CHO \xrightarrow{O} R \quad (CHOH)_n- COOH$$
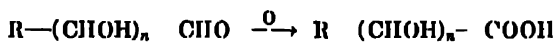
aldonic acids are valuable for characterization of the sugars. The preparation of an aldonic acid of the same number of carbon atoms has often been used as proof of aldehyde structure; the ketoses in contrast undergo chain splitting and form lower aldonic acids. The aldonic acids can also be reduced by HI to the corresponding aliphatic acids. A reducing disaccharide containing two dissimilar sugar units can be converted to the aldobionic acid; subsequent hydrolysis will give an aldonic acid of one monosaccharide and an aldose, and show the position of the reducing group in the original disaccharide.

The aldonic acids, especially gluconic acid in the form of soluble salts, are important to the pharmaceutical industry for the purpose of introducing appropriate metallic ions such as iron, bismuth and particularly calcium into the body in a neutral and easily assimilable form. Calcium lactobionate·CaBr₂ may have value as a sedative.

These acids are important precursors in the preparation of sugars with fewer carbon atoms. Oxidative degradation with H_2O_2 and iron salts (see p.

121) produces an aldose of one less carbon atom; thus, D-gluconic acid is converted to D-arabinose, and D-galactonic acid to D-lyxose. Nitriles and amides can also be degraded (see p. 122).

Methods for lengthening the carbon chains of sugars may involve the formation of aldonic acids as intermediates. The Kiliani cyanohydrin synthesis (see p. 116) creates two new aldonic acids with one more carbon atom than in the original aldose. The configuration of the new asymmetric atom can be assigned by use of the lactone rule discussed below.

Finally, an aldonic acid can be converted to its 2-epimer by the action of alkaline agents (see below).

The aldonic acids apparently do not occur naturally. Certain bacteria will oxidize glucose readily to gluconic acid, but no appreciable amount of such products have been found in plants or animals. Nature seems to prefer to manufacture unstable intermediates which can be converted easily to "metabolic products," and the formation of a stable substance like gluconic acid may be a stopping point in the reaction chain. In nature, the reactions are generally developed toward the oxidation of primary alcohol groups to form uronic acids or the splitting of carbon-carbon bonds in hexoses to trioses.

Apparently, gluconic acid and its salts are not metabolized but are excreted in the urine.[4] When the acid or salts is administered orally, only a small portion is absorbed as such, because of decomposition by microorganisms in the intestine. In proper amounts, gluconic acid and salts produce a decrease in the acidity of the urine.

a. PREPARATION

The synthesis of aldonic acids can be carried out in various ways. The methods involve not only the formation of a carboxyl group but frequently the creation or destruction of asymmetric carbon atoms. The methods given below are presented in a simplified manner, for side reactions and "over-oxidation" often occur.

Oxidation of an Aldose to the Corresponding Aldonic Acid. Bromine or nitric acid are the main oxidants, the latter under mild conditions. The best yields are obtained by the use of bromine in a slightly acid buffered solution (pH 5–6) (see p. 321). The products are generally isolated as the metallic salts by direct crystallization from the reaction solution or by precipitation into ethanol. Yields as high as 95% have been reported in the case of glucose. Commercially the indirect use of bromine as an oxidant is employed in the electrolytic oxidation process with calcium bromide as a "catalyst";

the constant regeneration of free bromine in the solution allows a very economical operation. In the case of rhamnose, the oxidation product can be isolated directly as the lactone; this is one of the few cases for which recourse to metallic salts is not necessary.

Oxidative Degradation. In this type of synthesis one or more asymmetric carbon atoms is lost and several related sugars may give the same product. Fructose and glucose can be oxidized with oxygen in alkaline solution to give a 70% yield of sodium D-arabonate.[s] L-Arabinose gives 40% of L-erythronic acid.[b] In such alkaline solutions the formation of enols is undoubtedly important. L-Ascorbic acid, an enediol, has been oxidized by sodium hypoiodite and by potassium permanganate to L-threonic acid.[7] Such oxidation of double bonds does not occur in the enols alone, for D-arabinal is oxidized by H_2O_2 and OsO_4 in *tert*-butanol to D-erythronic acid in addition to D-arabinose.[8] Periodic acid and lead tetraacetate are useful for the cleavage of hexitols and glycosides to glyceric and glycolic acids (see Chapter III).

Synthesis From Lower Aldoses. The Kiliani cyanohydrin synthesis has been discussed elsewhere (see p. 116). In this method a new asymmetric center is created, and two epimeric acids are formed in varying amounts. Because of the asymmetric nature of the original sugar, the proportion of epimers formed is rarely if ever in the ratio 1:1.

Change of Configuration Without Change in Number of Carbon Atoms. Epimerization of carbon 2 of an aldonic acid can be carried out in the presence of alkaline agents. This reaction is discussed later.

Synthesis of Acids from Noncarbohydrates. This reaction is a specialized one utilized in the synthesis of tetronic acids, because of the rarity of the tetroses: threose and erythrose. Thus, the oxidation of 3-chlorocrotonic acid with OsO_4 and $Ba(ClO_3)_2$ followed by the action of Ag_2O gives D,L-threonic acid (for details, see Chapter III).

b. EQUILIBRIUM IN SOLUTION

The free aldonic acids seldom exist as such in aqueous solution; instead they readily form lactones (inner esters) by elimination of water as shown below. Either of the hydroxyls in the γ- and δ-positions can take part in this reaction. The δ-lactones usually hydrolyze easily and mutarotate rapidly in aqueous solution. In contrast, the γ-lactones are more stable and are converted only slowly in water to the equilibrium mixture of free acid and lac-

[s] O. Spengler and A. Pfannenstiel, *Z. Ver. deut. Zucker-Ind.*, **85**, 546 (1933).

[b] J. U. Nef, O. F. Hedenburg and J. W. E. Glattfeld, *J. Am. Chem. Soc.*, **39**, 1638 (1917).

[7] R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. W. Reynolds and F. Smith, *J. Chem. Soc.*, 1270 (1933)

[8] R. C. Hockett and S. R. Millman, *J. Am. Chem. Soc.*, **63**, 2587 (1941).

tones. In Fig. 1 is shown the mutarotation of several methylated lactones.[*] The distinction between the two types of lactones is very evident.

$$
\begin{array}{ccc}
\begin{bmatrix}
C=O \\
HCOH \\
HOCH \\
HCOH \\
HC
\end{bmatrix} O
&
\begin{array}{c}
COOH \\
HCOH \\
HOCH \\
HCOH \\
HCOH
\end{array}
&
\begin{bmatrix}
C=O \\
HCOH \\
HOCH \\
HC \\
HCOH
\end{bmatrix} O \\
CH_2OH & CH_2OH & CH_2OH
\end{array}
$$
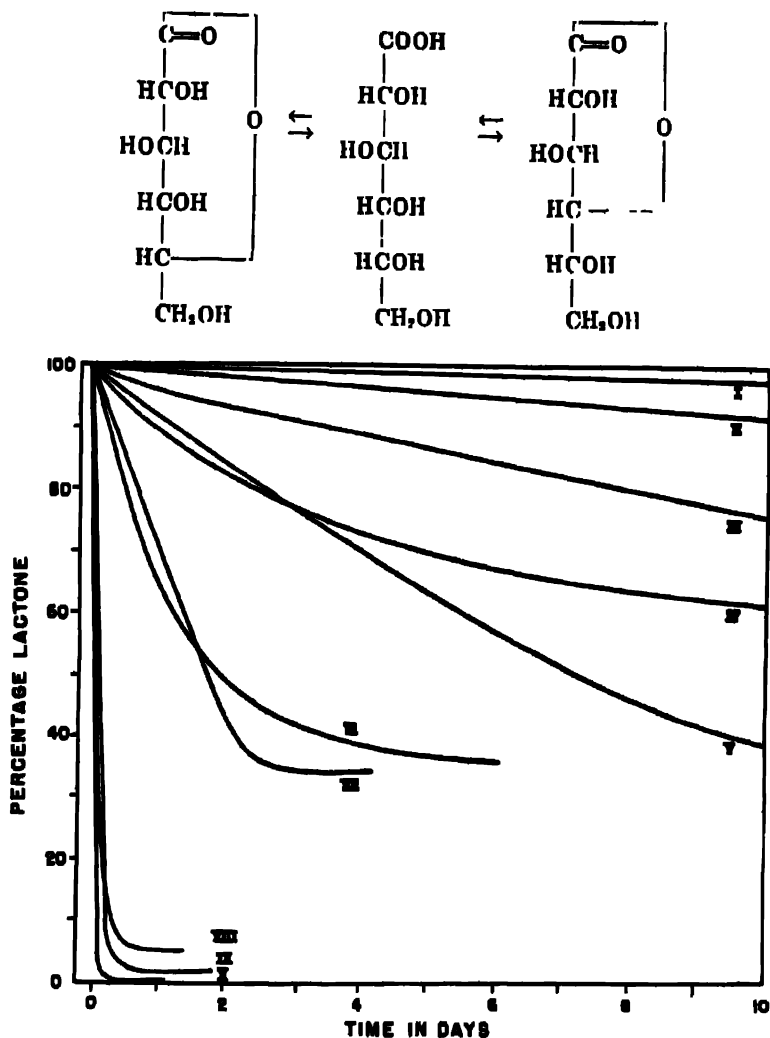
Fig 1 Mutarotation of methylated lactones   (After Haworth)

I Tetramethylmannonic γ lactone	VI Tetramethylmannonic δ lactone
II Tetramethylgalactonic γ-lactone	VII Trimethylxylonic δ-lactone
III Trimethylxylonic γ-lactone	VIII Tetramethylgluconic δ lactone
IV Tetramethylarabonic γ-lactone	IX Tetramethylgalactonic δ lactone
V. Tetramethylgluconic γ-lactone	X Trimethylarabonic δ-lactone

[*] W. N. Haworth, "The Constitution of Sugars," p 24; Edward Arnold, London (1929)

Solutions of aldonic acids or lactones equilibrate to mixtures of the free acid and the δ- and γ-lactones, the relative proportions of which depend upon the configuration of the asymmetric carbon atoms. The attainment of equilibrium conditions is reached only after many days at room temperature but is accelerated by the presence of strong acids.

For gluconic δ-lactone an initial rapid hydrolysis to a mixture consisting mainly of the free acid and δ-lactone occurs; subsequently a slow rise in rotation takes place until the value obtained approaches that found for the other two forms. The changes in the rotation of gluconic acid and its lactones are given in Table I.

Equilibrium solutions of acids and lactones of the mannose series contain large pr portions of γ-lactones, whereas those of the glucose series contain large proportions of the δ-lactones and free acids. The lactone of D-gluco-

TABLE I

Optical Rotations of Gluconic Acid and Lactones[10]

Carbohydrate	Initial Rotation $[\alpha]_D$	Final Rotation $[\alpha]_D$	Time
D-Gluconic acid	−6.7	+17.5	10 days
D Gluronic γ-lactone	+67.5	+17.7	14 days
D-Gluconic δ-lactone	+66	+8.8	24 hours
		+11.5	95 hours
		+15.8	25 days

D-*ido*-heptonic acid mutarotates without an increase in acidity, and apparently little or none of the free acid is formed.

A solution supersaturated with respect to both free acid and lactone can often be seeded with the appropriate crystals and the desired product obtained. Normally, the free aldonic acid is obtained by concentration of the aqueous solution at a low temperature *in vacuo*. The free acid can also be crystallized from a solution of the sodium salt in acetic acid. The lactones are formed by dehydration, often very easily. Water can be removed by distillation with butanol or dioxane or by heating *in vacuo*. The lactones are crystallized from an anhydrous solvent; in some cases, as with rhamnonic γ-lactone, they are formed very easily and crystallize readily from water.

Solvents have a definite effect on the equilibrium composition. Thus, mannonic acid dissolved in acetic acid with 16% of water shows a higher positive rotation than in water. The mutarotation is slower, but there is apparently a greater conversion to the δ-lactone than to the γ-lactone. The

[10] H. S. Isbell and H. L. Frush, *J. Research Natl. Bur. Standards*, *11*, 649 (1933); J. U. Nef, *Ann.*, *403*, 322 (1914); O. F. Hedenburg, *J. Am. Chem. Soc.*, *37*, 345 (1915); H. S. Isbell and C. S. Hudson, *J. Research Natl. Bur. Standards*, *8*, 327 (1932).

pH, temperature and concentration also have an effect on the final equilibrium.

In addition to lactone formation, it is probable that extramolecular esterification may take place with the formation of aldonic esters of aldonic acids (*e.g.*, gluconic acid gluconate) and chain polymerization also may occur. In such systems, the concentration of water present would be expected to exert a profound influence on the composition of the equilibrium solution. Lactic acid forms external esters (lactides), but this type of condensation through carboxyls and α-hydroxyls has not been observed for hexonic and pentonic acids.

c. EPIMERIZATION

The aldonic acids, in contrast to the reducing sugars, are relatively stable under alkaline conditions. The configuration of carbon 2 can be altered, however, by prolonged heating with various alkaline agents. Gluconic acid, heated with barium hydroxide at 100° for 115 hours, is converted[11] to the 2-epimer (mannonic acid) in a yield of 20%. As the reverse reaction under the same conditions provides only 12% conversion to gluconic acid, the attainment of equilibrium is very slow. This type of interconversion was first[12] carried out with quinoline at 140°. Aqueous pyridine[13] produces 25% conversion of galactonic acid to talonic acid in 115 hours at 100°. Dibasic acids behave similarly; mucic acid is transformed to D L-talomucic acid.[13a]

It is interesting that this epimerization can occur when the hydroxyl on carbon 2 is methylated. Both tetramethylgluconic δ-lactone and tetramethylgluconic γ-lactone can be converted to the corresponding mannose derivatives.[14] The trimethylxylonic lactones are transformed to those with the lyxose configuration.

The epimerization may take place through an intermediate enediol as for the sugars. The epimerization of methylated derivatives might occur
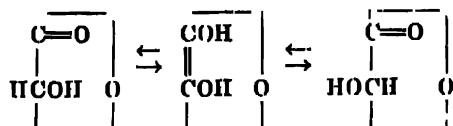
since the methoxyl on carbon 2 is not involved. One possible objection to this concept is that the postulated enediol is also the enediol of an osone which should yield the same products and which might be formed from

[11] H. T. Bonnett and F. W. Upson, *J. Am. Chem. Soc.*, *55*, 1215 (1933).

[12] E. Fischer, *Ber.*, *23*, 799 (1890); *24*, 2136 (1891).

[13] O. F. Hedenburg and L. H. Cretcher, *J. Am. Chem. Soc.*, *49*, 478 (1927).

[13a] T. Posternak, *Natui wissenschaften*, *23*, 287 (1935).

[14] W. N. Haworth and C. W. Long, *J. Chem. Soc.*, 345 (1929).

aldonic acids. No osones have been obtained from such reactions, but the compounds are very difficult to isolate.

d. Optical Rotatory Relationships

A number of empirical relationships between the optical rotations of acids, lactones, salts and derivatives have been derived. The most important use of these relationships is for the determination of the configurations of the epimeric acids produced in the cyanohydrin synthesis.

The configuration of the hydroxyl groups on carbons 4 and 5 has a major influence on the rotations of lactones. The "lactone rule" in its qualitative form[15] stipulates that a lactone is more dextrorotatory than the free acid if the hydroxyl group involved in lactone formation lies on the right side in the Fischer projectional formula. The lactone will be more levorotatory than the acid if the hydroxyl group lies on the left side. Since most aldonic acids have only small rotations, and the lactones, because of ring formation, possess fairly strong rotations, the lactones can be divided into levorotatory and dextrorotatory groups. Both γ- and δ-lactones of gluconic and mannonic acid are dextrorotatory; gulonic and galactonic acids form levorotatory γ-lactones and dextrorotatory δ-lactones. D-Allonic γ-lactone provides an exception to the rule since it has a small negative rotation ($[\alpha]_D$ −6.8) instead of the expected positive rotation.

The differences in rotation of pairs of γ-lactones epimeric at carbon 2 divides the lactones into two distinct classes:[16] those with molecular epimeric differences in the range −3400 to −4000 (ribonic, arabonic, galactonic, talonic and homomorphous lactones) and those with differences of a different sign (xylonic, lyxonic, gluconic and mannonic lactones).

The configuration of carbon 2 exerts a major influence on the rotation of acyclic derivatives of the aldonic acids. The phenylhydrazides and amides are dextrorotatory when the hydroxyl group on carbon 2 lies to the right in the Fischer projectional formula.[17] For these derivatives, gluconic and mannonic acid have rotations with different signs, whereas the derivatives of gluconic and galactonic acid have the same signs. The lactone and hydrazide rules are very valuable in the determination of configuration, especially of new aldonic acids formed by the cyanohydrin synthesis. These derivatives are generally used to characterize the acids and can also be employed for configurational identification. A similar rule also applies to the benzimidazole derivatives[18] and to the acetylated nitriles.[19]

[15] C. S. Hudson, *J. Am. Chem. Soc.*, **32**, 338 (1910); F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars," p. 434; Gov't. Printing Office, Washington (1942).

[16] C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 1525 (1939).

[17] C. S. Hudson, *J. Am. Chem. Soc.*, **39**, 462 (1917); **40**, 813 (1918).

[18] N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 1612 (1942).

[19] V. Deulofeu, *Nature*, **131**, 548 (1933).

Normally the alkali salts of the aldonic acids are slightly more dextro-rotatory than the free acids, when the hydroxyl of carbon 2 lies on the right.[20] This correlation might be considered to be an extension of the hydrazide rule. The lead salts present an exception, apparently because a complex is formed between the lead ion and the hydroxyl on carbon 2.[21] The lead salts are acidic in contrast to the normal type. The rotatory displacement in relation to the calcium salts is levorotatory when the hydroxyl of carbon 2 is on the right.

c. REACTIONS OF THE ALDONIC ACIDS

The aldonic acids show the reactions typical of aliphatic organic acids. Their aqueous solutions have a pH of 2 to 3. The free acids are soluble in water and slightly soluble in ethanol; they are more soluble in nonpolar solvents than the sugars, and less soluble than the lactones. Various salts can be formed and their utility depends upon the nature of the acid. Gluconic and galactonic acid, formed by the acidic oxidation of lactose, can be separated by the use of cadmium salts. Cadmium galactonate is less soluble in water than the gluconate; after removal of the former, the gluconic acid is isolated as the typical calcium salt. Some metallic salts are unstable; mercuric gluconate decomposes easily into free mercury, the mercurous salt, arabinose and carbon dioxide. The use of lead salts for separating epimeric acids is described on p. 117.

The nature of the cation may influence the reactivity of salts greatly.[22a] Thus, cadmium D-ribonate can be acetylated in 85% yield, but other salts give smaller yields: ammonium salt, 46%; potassium salt, 25%; calcium salt, 22%; and barium salt, 4%.

Esters of aldonic acids are prepared from δ-lactones, but not from γ-lactones, by reaction with alcohols in the presence of hydrogen chloride or of the free aldonic acid.[22b] But the acids may be recrystallized from boiling methanol without esterification taking place.[23] At the melting point, ethyl mannonate is converted to the γ-lactone with the loss of ethyl alcohol.

Toward alkali, the lactones are less reactive than the acids. A solution of free acid can be neutralized with calcium carbonate or barium benzoate. Sodium carbonate reacts with the δ-lactones and an excess of sodium hydroxide with the γ-lactones.

The amides of the aldonic acids can be formed readily by the action of

[20] P. A. Levene, *J. Biol. Chem.*, **23**, 145 (1915); P. A. Levene and G. M. Meyer, *ibid.*, **31**, 623 (1017).

[21] H. S. Isbell, *J. Research Natl. Bur. Standards*, **14**, 305 (1935).

[22a] K. Ladenburg, M. Tishler, J. W. Wellman and R. D. Babson, *J. Am. Chem. Soc.*, **66**, 1217 (1944).

[22b] See: O. F. Hedenburg, *J. Am. Chem. Soc.*, **37**, 345 (1915)

[23] K. Rehorst, *Ber.*, **63**, 2279 (1930).

liquid ammonia on the lactones.[24] These derivatives are often crystalline and are useful for the characterization of the acids. The phenylhydrazides, prepared by reaction of acids or lactones with phenylhydrazine, can be converted to the free acids or lactones. Hydrolysis of hydrazides by alkalies is often slow or incomplete. Boiling copper sulfate solution gives a 90% yield of mannonic lactone, and the phenylhydrazine is oxidized to benzene and nitrogen.[25] Nitrous acid has been used to convert hydrazides to the lactones.[26]

$$
\begin{array}{ccccc}
\text{OCNHNH}_2 & & \text{OCN}_1 & & \text{OC} \text{---} \text{-} \\
| & \xrightarrow{\text{HNO}_2} & | & \to & | \qquad | \\
\text{HCOH} & & \text{HCOH} & & \text{HCOH} \quad \text{O} \\
| & & | & & | \qquad |
\end{array}
$$
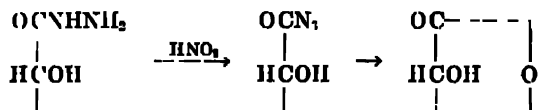
The aldonyl chlorides can be prepared[27] by treatment of acetylated aldonic acids with PCl$_5$. These chlorides are used for the preparation of open-chain derivatives of aldoses by catalytic reduction with hydrogen in xylene solution.[28] Keto acetates with one carbon atom more than the aldonyl chloride are formed by the action of diazomethane. Acetic acid removes the diazo group. In this manner L-fructose was made from L-arabonic acid.[29]

$$
\begin{array}{ccccccc}
\text{Cl} & & \text{CHN}_2 & & \text{CH}_2\text{OH} \\
| & & | & & | \\
\text{C}=\text{O} & \xrightarrow{\text{CH}_2\text{N}_2} & \text{C}=\text{O} & \xrightarrow[\text{Cu(OAc)}_2]{\text{HOAc}} & \text{C}=\text{O} & +\text{N}_2 \\
| & & | & & | \\
\text{HCOAc} & & \text{HCOAc} & & \text{HCOAc} \\
| & & | & & |
\end{array}
$$
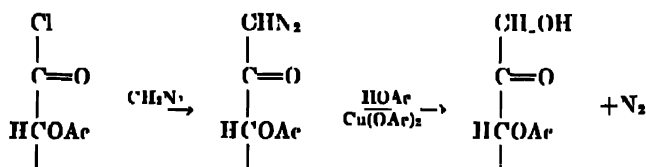
The action of HBr on the diazo compound is similar to that of acetic acid and a 1-bromo keto acetate is formed. Silver oxide causes a rearrangement to a 2-desoxy aldonic acid.[30]

$$
\begin{array}{cccc}
\text{CHN}_2 & & \text{COOH} \\
| & & | \\
\text{C}=\text{O} & \xrightarrow[\text{Ag}_2\text{O}]{+\text{H}_2\text{O}} & \text{CH}_2 & +\text{N}_2 \\
| & & | \\
\text{HCOAc} & & \text{HCOAc} \\
| & & |
\end{array}
$$
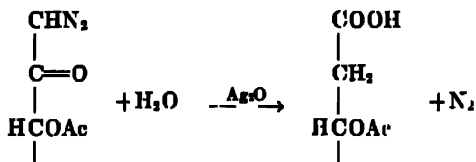
[24] J. W. E. Glattfeld and D. Macmillan, J. Am. Chem. Soc., 56, 2481 (1934).

[25] R. M. Hann and C. S. Hudson, J. Am Chem. Soc , 56, 957 (1934).

[26] A. Thompson and M. L. Wolfrom, J. Am. Chem. Soc., 68, 1509 (1946)

[27] R. T. Major and E. W. Cook, J. Am. Chem. Soc., 58, 2477 (1936); M. L. Wolfrom, R. L. Brown, and E. F. Evans, ibid , 65, 1021 (1943).

[28] E. W. Cook and R. T. Major, J. Am. Chem Soc., 58, 2410 (1936).

[29] M. L. Wolfrom and A. Thompson, J. Am. Chem. Soc., 68, 791 (1946).

[30] M. L. Wolfrom, S. W. Waisbrot and R. L. Brown, J. Am. Chem. Soc., 64, 1701, 2329 (1942).

Reduction of thioesters to aldoses can be carried out by catalytic hydrogenation methods. (See also p. 117.) Thus, ethyl thiol-D-ribonate tetraacetate gives aldehydo-D-ribose tetraacetate.[31]

$$\begin{array}{c}
\text{Cl} \\
| \\
\text{C}{=}\text{O} \quad + \text{ RSH} \\
| \qquad\quad + \text{ pyridine} \\
\text{HCOAc} \\
|
\end{array}
\longrightarrow
\begin{array}{c}
\text{SR} \\
| \\
\text{C}{=}\text{O} \quad + \text{ pyridine}\cdot\text{HCl} \\
| \\
\text{HCOAc} \\
|
\end{array}$$

$$\begin{array}{c}
\text{SR} \\
| \\
\text{C}{=}\text{O} \quad + \text{ 2H}_2 \\
| \\
\text{HCOAc} \\
|
\end{array}
\longrightarrow
\begin{array}{c}
\text{H} \\
| \\
\text{C}{=}\text{O} \quad + \text{ RH } + \text{ H}_2\text{S} \\
| \\
\text{HCOAc} \\
|
\end{array}$$
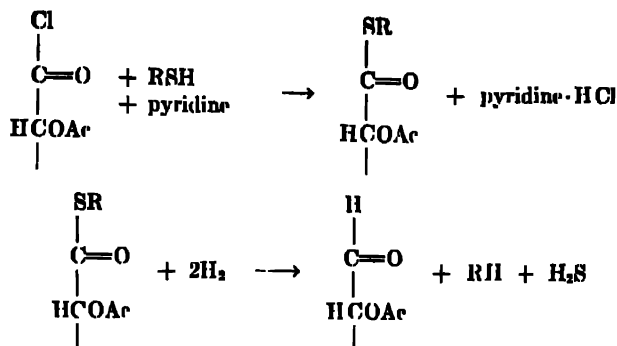
By catalytic hydrogenation, aldonic esters and lactones are reduced to glykitols.[32] The reduction of lactones to sugars by sodium amalgam was introduced by Fischer and has been extensively employed for the purpose (see Chapter III). Esters, but not the free acids, are reducible. In order to obtain maximal yields, the acidity must be maintained in the range 3 to 3.5. The temperature should be kept below 15°, and a minimum of 2.5 equivalents of sodium are required (theory, 2).[33]

B. Saccharic (Aric) Acids.[34] The saccharic acids are polyhydroxy dicarboxylic acids, $HOOC{-}(CHOH)_n{-}COOH$, and are generally obtained from the sugars by the action of strong oxidizing agents. Several of these acids, tartronic, erythraric, xylaric, allaric and galactaric, are optically inactive. The acid salts are often used for characterization, because of their low solubility in water. Mannaric and glucaric acids show abnormal behavior in alkaline solution, with rearrangement to enolic forms. Commercially, the acids, especially threaric and glucaric, have been utilized for the preparation of salts of therapeutical importance.

a. TARTRONIC AND MALIC ACID

Tartronic acid, $HOOC{-}CH(OH){-}COOH$, or hydroxymalonic acid, may be considered as the simplest of the aric acids. It has been obtained by the oxidation of glucose or fructose with hydrogen peroxide and ferrous sul-

[31] M. L. Wolfrom and J. V. Karabinos, J. Am. Chem. Soc., 68, 1455 (1946).

[32] J. W. E. Glattfeld and A. M. Stark, J. Am. Chem. Soc , 59, 753 (1937).

[33] N. Sperber, H E Zaugg and W. M. Sandstrom, J. Am. Chem. Soc., 69, 915 (1947).

[34] The term "aric" is used with the normal configurational prefix; the tartaric acids are threaric or erythraric acids, mucic acid is galactaric acid, and gluco-saccharic acid is glucaric acid. The name xylaric acid is much shorter than xylo-trihydroxyglutaric acid. For an additional discussion see below and Chapter I, particularly Table V. In the present text both forms are used, but the new usage is preferred.

late.[35] It is also formed by the cyanohydrin synthesis from glyoxylic acid.[36] The oxidation of glycerol gives only small amounts of this acid.

$$HOOC—CHO + HCN \rightarrow HOOC—CH(OH)--CN \rightarrow HOOC—CH(OH)- COOH$$

Malic acid, $HOOC$ CH_2 $CH(OH)$ $COOH$, may be considered as a desoxytetraric (tartaric) acid. It occurs widely in nature in fruits and berries. It is formed by the partial reduction of tartaric acids with HI or by the addition of the elements of water to fumaric or maleic acid. The natural acid is levorotatory in dilute solutions, but the rotation becomes positive with increasing concentration. This effect has also been noticed with L-tartaric (L-threaric) acid.

b. TETRARIC ACIDS (TARTARIC ACIDS)[37]

These acids exist in four forms:

> L-Threaric acid (L-tartaric acid)
> D-Threaric acid (D-tartaric acid)
> D,L-Threaric acid (D,L-tartaric or racemic acid)
> Erythraric acid (*meso*-tartaric acid).

L-Threaric acid occurs naturally as the mono potassium salt, especially in the juice of grapes. The sodium-potassium salt ($NaKC_4H_4O_6$ $4H_2O$) is known as Rochelle salt and the potassium-antimonyl salt ($K(SbO)C_4H_4O_6$ $\frac{1}{2}H_2O$) as tartar emetic. The D-acid can be obtained from the racemic mixture by resolution of the cinchonine salts.[38] The D-gluco-D-*gulo*-heptobenzimidazole forms a salt with L-threaric acid that allows of the resolution of the D,L-form.[39] Pasteur originally resolved this form by mechanical separation of crystals of the sodium-ammonium salt.

The D,L-racemate and the inactive isomer are formed from the L-acid by heating with water at 150° to 170°. Heating with alkali has the same effect, but the yields of the two products vary according to conditions.[40] Separation is effected on the basis of the much greater solubility of the potassium hydrogen salt of the meso acid (8% in water at 19°) compared with that of the racemic acid (0.5% in water at 19°). Oxidation of fumaric acid with chlorates and OsO_4 produces the D,L-form, whereas the meso isomer is obtained from maleic acid.[41]

⁶ C F Cross, E J. Bevan and C. Smith, *J. Chem. Soc.*, *73*, 160 (1898).

³⁶ C. Böttinger, *Ber.*, *14*, 729 (1881)

³⁷ The common form of designation of these acids is to use *d* and *l* for the sign of rotation rather than L and D, respectively, for indications of configuration. For further discussion, see p 40.

³⁸ W. Markwald, *Ber.*, *30*, 42 (1897).

³⁹ W. T. Haskins and C. S. Hudson, *J. Am. Chem. Soc*, *61*, 1266 (1939).

⁴⁰ See: "Organic Syntheses," Collective Volume I, 484 (1932); "Beilsteins Handbuch der organischen Chemie," vol *3*, p. 528 (1921)

⁴¹ N. A. Milas and E. M. Terry, *J. Am. Chem. Soc.*, *47*, 1412 (1925); G. Braun, *ibid.*, *51*, 247 (1929).

The optical rotation of L-threaric acid in water is positive at high concentrations but drops with dilution and finally becomes negative. Complex formation with salts, borates and molybdates affects the optical rotation greatly. Rotational values in alcohols are very low.

The heating of L-threaric acid above 100° forms an anhydride; initially, gummy materials are formed as a result of external condensation and finally at 170° an insoluble anhydride is produced.

The solubility of the monopotassium salt of the D,L-racemic acid differs little from that of the L-acid, but the solubility of the calcium salts differs sufficiently to allow a separation.[42]

The tartaric acids are formed by the oxidation of hexose sugars and of the keto acids. (See under nitric acid and alkaline oxygen oxidations, particularly.) The L-isomer has been recovered from grape residues by concentration on a basic ion-exchange resin.[43]

c. Pentaric and Hexaric Acids

The four pentaric acids and ten hexaric acids are:

Pentaric (Hydroxyglutaric) Acids

Xylaric (meso)	= xylo-trihydroxyglutaric
Ribaric (meso)	= ribo-trihydroxyglutaric
D- and L-Arabaric	= D- and L-lyxaric
	= D- and L-arabo-trihydroxyglutaric

Hexaric Acids

D- and L-Mannaric	= D- and L-manno-saccharic
D- and L-Glucaric	= D- and L-gluco-saccharic
	= L- and D-gularic
D- and L-Idaric	= D- and L-ido-saccharic
D- and L-Talaric	= D- and L-talomucic
	= D- and L-altraric
Allaric (meso)	= allomucic
Galactaric (meso)	= mucic

The pentaric (hydroxyglutaric) acids are important primarily as reference compounds in structural proofs. They can be prepared by oxidation of the corresponding pentoses with nitric acid.

Several of the hexaric acids are of especial interest. Galactaric (mucic) acid has a low solubility in water, and its formation by the nitric acid oxidation of galactose is used for the quantitative determination of galactose. Its formation by bromine oxidation is considered satisfactory evidence of the presence of galacturonic acid. The acid can be prepared on a large scale

[42] A. Holleman, Rec. trav. chim., 17, 69 (1898); J. M. Albahary, Compt. rend., 144, 1232 (1907).
[43] J. R. Matchett, Ind. Eng. Chem., 36, 851 (1944).

by the nitric acid oxidation of galactans prepared from certain woods.[44] It is interesting that acetylation increases the solubility of galactaric acid in water. Ammonium galactarate (mucate) forms pyrrole when heated.

In contrast to galactaric acid, D-mannaric and D-glucaric acids are appreciably soluble in water. Glucaric acid is best prepared by the nitric acid oxidation of starch; yields as high as 65% are obtained in contrast to much lower yields from glucose or sucrose.[45] This acid is generally characterized as the potassium acid salt or silver salt

The saccharic acids do not reduce Fehling solution but will react with ammoniacal silver nitrate. However, the dilactones of mannaric and glucaric acids show an unexpected reducing action with Fehling solution.[46] This same behavior is shown with the monoester monolactones of glucaric acid. The monolactones do not show this behavior. The alkali cleaves the lactone ring and the necessary hydrogen atom is provided from the neighboring carbon atom rather than from the solution. Uronic acid lactones
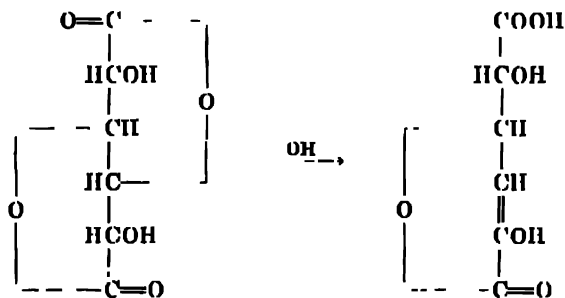
behave similarly. The resulting enol is the enolic lactone of a 4-desoxy 5-keto dibasic acid related to the ascorbic acids. These enols react with only a small amount of iodine, in contrast to the behavior of the ascorbic acids. However, four atoms of chlorine are taken up, whereas the ascorbic acids react with only half of this amount. In alkaline solution ozone attacks the double bond, forming oxalic acid and either erythruronic or threuronic acid.

The saccharic acids can be used as starting materials for other carbohydrate products. Epimerizations can be carried out with pyridine as with the aldonic acids. Galactaric acid is converted to D,L-talaric acid. The two monolactones of D-glucaric acid are reduced by sodium amalgam to different products. The 3,6-lactone (IV) forms L-guluronic (V) and D-gluconic (VI) acids, and the 1,4-lactone (I) forms D-glucuronic (II) and L-gulonic (III) acids.[47] The two lactones can be obtained from glucaric acid solutions by seeding with the proper nuclei.

[44] A. W. Schorger, U. S. Patent 1,718,837, June 25, 1929.
[45] See H. Kiliani, Ber., 58, 2344 (1925); O. T Schmidt, H. Zeiser and H. Dippold, ibid., 70, 2402 (1937).
[46] See: F. Smith, Advances in Carbohydrate Chem., 2, 101 (1946).
[47] M. Sutter and T. Reichstein, Helv. Chim. Acta, 21, 1210 (1938).

```
O=C ---┐          CHO              COOH
  |     |           |                |
HCOH    |         HCOH             HOCH
  |     |O          |                |
HOCH    |    →    HOCH      →      HOCH
  |     |           |                |
HC- ---┘         HCOH             HCOH
  |                |                |
HCOH             HCOH             HOCH
  |                |                |
COOH             COOH             CH₂OH
 (I)              (II)             (III)
```

```
    COOH           COOH             COOH
     |              |                |
   HCOH           HCOH             HCOH
     |              |                |
    CH      →    HOCH       →     HOCH
 |   |            |                |
 |  HCOH         HCOH             HCOH
 O   |            |                |
    HCOH         HCOH             HCOH
     |            |                |
    C=O          CHO             CH₂OH
    (IV)          (V)             (VI)
```
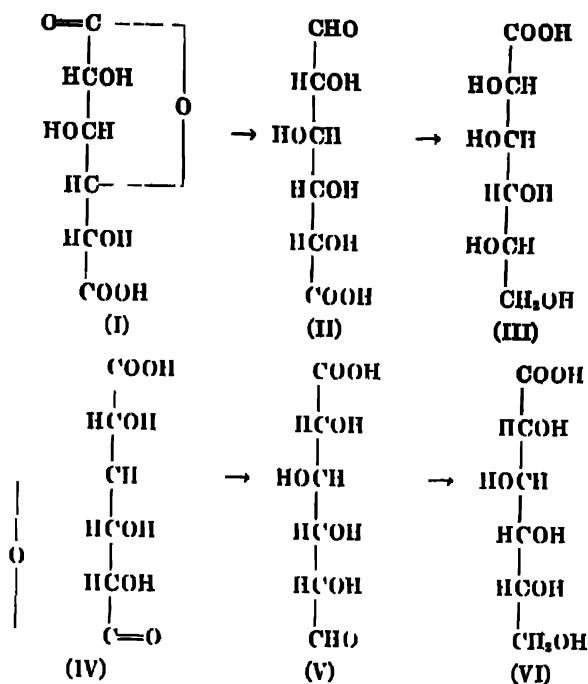
C. Uronic Acids.

a. PREPARATION AND OCCURRENCE

The uronic acids may be defined as carbohydrate derivatives possessing both aldehyde (or hemiacetal) and carboxyl groups. The formulas for the three naturally occurring acids are given below

```
  CHO            CHO              CHO
   |              |                |
 HCOH           HOCH            HCOH
   |              |               |
 HOCH           HOCH            HOCH
   |              |               |
 HCOH           HCOH            HOCH
   |              |               |
 HCOH           HCOH            HCOH
   |              |               |
 COOH           COOH            COOH
```
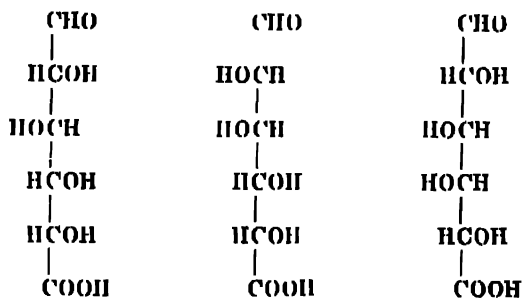
D-Glucuronic acid   D-Mannuronic acid   D-Galacturonic acid

The uronic acids biologically are very important. As shown in Table II, they occur as important building units in many polysaccharides particularly pectins and alginic acid (Chapters XII and XV). One, glucuronic acid, serves as a detoxifying agent in mammals, and some poisonous substances are eliminated in the urine as glucuronides (see Chapter XI).

The isolation of uronic acids from polysaccharides is not easy. Some of the linkages are very resistant to acid hydrolysis. Sulfuric ac.d (4%) at 120° for 10–24 hours[48] is often required. This harsh treatment may decompose the products considerably, and the yields are generally low. Cold 80% sulfuric acid[49] and 3% oxalic acid[50] at 100° have been used for the hydrolysis of alginic acid. In the pectin field, enzymatic hydrolysis has been used for the isolation of galacturonic acid; the procedure is very mild and excellent yields are obtained.

<div align="center">

TABLE II

Natural Occurrence of Uronic Acids

</div>

D-Glucuronic Acid
  1. Urine of animals.
  2. Mucopolysaccharides (see Chapter XV).
      Heparin (with D-glucosamine and sulfates)
      Chondroitin sulfate (with N-acetylchondrosamine and sulfates)
      Hyaluronic acid (with N-acetyl-D-glucosamine).
      Type II pneumococcus specific polysaccharide (with glucose and rhamnose)
      Type III pneumococcus specific polysaccharide (with glucose)
      Type VIII pneumococcus specific polysaccharide (with glucose)
      Azotobacter and *Rhizobia* capsular polysaccharides (with glucose)
      Friedländer's bacillus polysaccharides (with glucose)
      Cytophagae polysaccharide (with glucose)
  3 Gum arabic
  4. Saponins, glycosides and oligosaccharides of certain types
  5. Various woods as monomethyl ethers (?)
D-Galacturonic acid
  1. Pectins and pectic acid.
  2. Type I pneumococcus specific polysaccharide.
  3. Flax seed mucilage and mucilage of slippery elm
D-Mannuronic Acid
  1. Alginic acid from sea weeds, as the sole constituent

Two general methods for the synthesis of uronic acids have been developed: (1) the reduction of the monolactones of saccharic acids, and (2) the oxidation of primary alcoholic groups of sugars or derivatives. The monolactones of dibasic acids can be reduced by sodium amalgam in acid solution. *gluco*-Saccharic acid was converted to glucuronic acid, but the maximal yield was 20%.[51] This method was later applied to the reduction of *manno*-saccharic acid to mannuronic acid, and of allomucic acid to the corresponding uronic acid.[52]

[48] E. Anderson, F. H. Russell and L. W. Seigle, *J. Biol. Chem*, *115*, 683 (1936).
[49] C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, *51*, 1914 (1929)
[50] W. A. G. Nelson and E. G. V. Percival, *J. Chem. Soc*, 58 (1942).
[51] E. Fischer and O. Piloty, *Ber*, *24*, 522 (1891).
[52] C. Niemann and K. P. Link, *J. Biol Chem.*, *100*, 407 (1933); C. Niemann, S Karjala and K. P. Link, *ibid.*, *104*, 189 (1934).

$$\begin{array}{ccc}
\text{OC} & & \text{CHO} \\
| & & | \\
\text{(HCOH)}_3 \quad \text{O} & \xrightarrow{\text{Na—Hg}} & \text{(HCOH)}_4 \\
| & & | \\
\text{HC—} & & \text{COOH} \\
| & & \\
\text{HCOH} & & \\
| & & \\
\text{COOH} & &
\end{array}$$
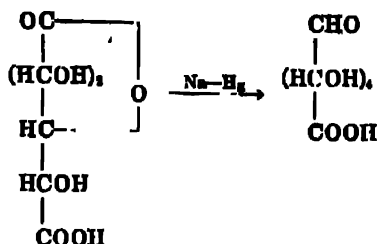
Unsubstituted primary alcoholic groups of derivatives of sugars have been oxidized to carboxyl groups. Glucuronic acid has been prepared from 1,2-isopropylidene-3,5-benzylidene-glucofuranose by the action of alkaline permanganate and the subsequent removal of substituent groups.[53] The oxidation of 1,2,3,4-tetraacetylglucose with permanganate in acetic acid serves a similar purpose.[54] 1,2,3,4-Diisopropylidene-galactose[55] can be oxidized with alkaline permanganate or the 1,2,3,4-tetraacetate can be used as in the case of glucose. For the preparation of D-mannuronic acid,[56] methyl 2,3-isopropylidene-α-D-mannoside has been oxidized with alkaline permanganate or the methyl 2,3,4-triacetyl-α-D-mannoside with permanganate in acetic acid. In the first instance, the unprotected hydroxyl on carbon atom four is not attacked.

Methyl α-D-mannopyranoside has been oxidized with Ba(OBr)₂ at 3° for 16 to 20 days and a 12% yield of methyl α-mannuronide obtained.[57] Methyl α-glucopyranoside has been converted[58] to the uronide in yields as high as 30% by the action of hydrogen peroxide with ferric salts as catalysts. Several other oxidations of glycosides have been reported, but the yields were very low. Oxidations with nitrogen dioxide seem particularly suited for this purpose (see under Nitric acid oxidations).

Uronic acids of the pentose series have been prepared by the oxidative degradation of amides. Mucic acid monoamide can be converted by the action of hydrogen peroxide and iron salts or by hypobromite to the corresponding lyxuronic acid.[59] The acids were isolated as the phenylosazone-phenylhydrazides or as the tetraacetates of the amide.

The action of bacteria on glucose leads to the formation of an easily reducing acid, tentatively identified as L-guluronic acid.[60] It would seem

[53] L. Zervas and P. Sessler, *Ber.*, *66*, 1326 (1933).
[54] M. Stacey, *J. Chem. Soc.*, 1529 (1939).
[55] H. Ohle and Gertrud Berend, *Ber.*, *58*, 2585 (1925).
[56] R. G. Ault, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 517 (1935); M. Stacey and P. I. Wilson, *ibid.*, 587 (1944).
[57] E. L. Jackson and C. S. Hudson, *J. Am. Chem. Soc.*, *59*, 994 (1937).
[58] K. Smolenski, *Rocsniki Chem.*, *3*, 153 (1924).
[59] M. Bergmann, *Ber.*, *54*, 1362 (1921).
[60] K. Bernhauer and K. Irrgang, *Biochem. Z.*, *280*, 360 (1935).

that microbial oxidation should receive additional study as a method for the preparation of uronic acids.

The identification of the uronic acids is difficult.[61] The alkaloidal salts frequently are used; cinchonine and brucine have value for glucuronic acid. Various hydrazines have been used to prepare derivatives, but often the products are complex, for hydrazides, hydrazones and osazones are formed. A common method of identification is to convert the uronic acids by mild oxidation to the dibasic acids.

When hexuronic acids are boiled with strong acids and naphthoresorcinol a blue color is formed. This reaction has been developed into a quantitative method.[62] The coloring matter formed is extracted with benzene and determined photometrically

b. ALDOBIURONIC ACIDS

An aldobiuronic acid (I)[63] may be defined as a disaccharide in which one of the sugar components is a uronic acid linked in glycosidic union to a hexose or pentose unit. Conceivably a disaccharide could exist which would contain a uronic acid unit with a glycosidic linkage at the hexose or pentose portion, as in II below, but compounds of this type are not known at present.

Aldobiuronic acids are readily isolated because of the strong resistance of the biuronic linkage to acid hydrolysis. Whereas 4% acid at 100–120° is

(I) Aldobiuronic Acid　　　　　　　(II) Pseudo-aldobiuronic Acid
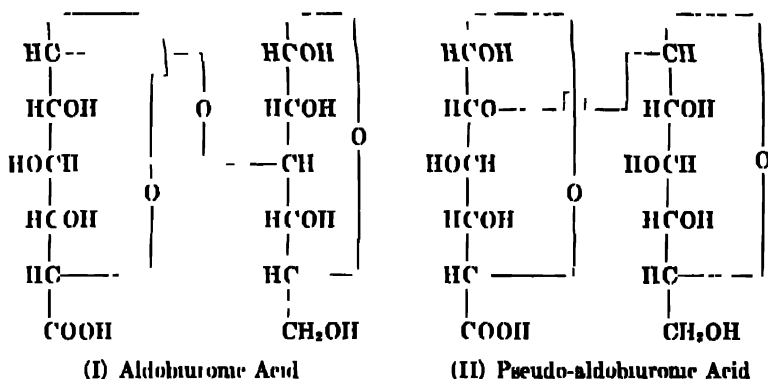
[61] See E Anderson and L Sands, *Advances in Carbohydrate Chem*, *1*, 329 (1945); M Stacey, *ibid.*, *2*, 170 (1946).

[62] See: S. W F. Hanson, C. T Mills and R T Williams, *Biochem J.*, *38*, 274 (1944); E. M. Knapp, *J Biol. Chem.*, *134*, 145 (1940).

[63] The term *aldobionic acid* is prevalent, but its use for this type of compound is not a happy choice, for it may be confused with the names of aldonic acids of disaccharides such as cellobionic acid

often used for the isolation of uronic acids, O'Dwyer[68] isolated an aldobiuronic acid from oakwood hemicellulose by the action of 1% sulfuric acid at 100°. This resistance to hydrolysis may explain the occurrence of uronides in soil. Some 10-15% of the organic carbon in surface soil appears to be combined uronic acids, and the amount increases with the depth of the soil.[64]

TABLE III

Sources of Aldobiuronic Acids

Name	Source
A. Aldobiuronic Acids from Bacterial Polysaccharides	
Glucuronosylglucose[65] [66]	Type III pneumococcus specific polysaccharide
Glucuronosylglucose[67]	Type A Friedlander's bacillus
6-Glucuronosylglucose[68] (Gentiobiuronic acid)	Synthetic
B. Aldobiuronic Acids from Gums and Woods	
2-Galacturonopyranosylrhamnose[68] [69]	Flaxseed mucilage
6-β-Glucuronosylgalactose[70-74]	Gum arabic (gum acacia)
Galacturonosylrhamnose[75]	Mucilage of slippery elm
Acid composed of xylose and methyluronic acid[76]	Oakwood
Acid composed of xylose and glucuronic acid[77]	Cottonseed hulls
Aldotriuronic acid composed of two xylose and one methyluronic acid units[78]	Cottonwood
Acids composed of one to three galactose and one methylglucuronic acid units[79]	Mesquite gum

[68a] M. H. O'Dwyer, *Biochem. J.*, *28*, 2116 (1934)

[64] A. G. Norman and W. V. Bartholomew, *Soil Sci.*, *56*, 113 (1943).

[65] M. Heidelberger and W. F. Goebel, *J. Biol. Chem.*, 74, 613 (1927)

[66] R. D. Hotchkiss and W. F. Goebel, *J. Biol. Chem.*, 115, 285 (1936)

[67] M. Heidelberger and W. F. Goebel, *J. Biol. Chem.*, 74, 619 (1927)

[68] E. Anderson and J. A. Crowder, *J. Am. Chem. Soc.*, *52*, 3711 (1930)

[69] R. S. Tipson, C. C. Christman and P. A. Levene, *J. Biol. Chem.*, *128*, 609 (1939)

[70] M. Heidelberger and F. E. Kendall, *J. Biol. Chem.*, 84, 639 (1929)

[71] C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, *51*, 1519 (1929)

[72] S. W. Challinor, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 258 (1931)

[73] W. F. Goebel and R. E. Reeves, *J. Biol. Chem.*, *124*, 207 (1938)

[74] P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, 125, 345 (1938)

[75] E. Anderson, *J. Biol. Chem.*, 104, 163 (1934)

[76] M. H. O'Dwyer, *Biochem. J.*, *28*, 2116 (1934)

[77] M. H. O'Dwyer, *Biochem. J.*, *20*, 664 (1926)

[78] E. Anderson, R. B. Kaster and M. G. Seeley, *J. Biol. Chem.*, *144*, 771 (1942)

[79] E. Anderson and L. Otis, *J. Am. Chem. Soc.*, *52*, 4461 (1930)

In Table III the various known aldobiuronic acids are listed. Wood hemicelluloses, plant mucilages, gums, and bacterial polysaccharides provide the natural sources. In addition several have been synthesized. Extreme interest has been evidenced in the bacterial products because of their relationship to immunological properties. The most thorough work on structure has been done on the acid obtained from gum arabic.

Aldobiuronic acids represent the penultimate stage of hydrolysis of the polyuronides. The action can be stopped at earlier stages. Aldotriuronic acids have been obtained. From mesquite gum, acids representing several stages of hydrolysis were isolated.[79] The aldobiuronic acid contained a galactose and a methylglucuronic acid unit. At lesser degrees of hydrolysis two or three galactose units were present; products of still slighter extents of hydrolysis contained four units of L-arabinose and three of galactose in addition to the uronic acid.

Oxidation of an aldobiuronic acid with bromine under nonhydrolytic conditions produces a dibasic acid in which the new carboxyl is formed from the original hexose or pentose unit. This is shown by the fact that such an acid (when the reducing portion of the original biuronic acid is a hexose) will form the same amount of furfural as the original acid under the action of 12% HCl. Evidently, the glycosidic linkage is formed from the hemiacetal group of the uronic acid. Oxidation with bromine under hydrolytic conditions produces a dibasic and an aldonic acid and allows identification of the two units.

c. REACTIONS OF URONIC ACIDS

One of the most important reactions observed with uronic acids is the decarboxylation caused by heating with strong acids (usually about 12% hydrochloric acid). The quantitative evolution of one mole of carbon dioxide was first observed by Tollens and Lefevre[80] and has been developed as an analytical method by many workers. The formation of the carbon dioxide is quantitative according to the following equation

$$C_6H_{10}O_7 \cdot C_5H_4O_2 + CO_2 + 3 H_2O$$

The liberation of carbon dioxide has also been observed for nonuronic carbohydrates, but the evolution is generally very slow.[81]

The mechanism of the decarboxylation is not well known. The above equation is not entirely correct, for the maximal yield of furfural ($C_5H_4O_2$) is only about 40% It is unlikely that the reaction proceeds through the formation of a pentose. Pentoses have never been isolated from such a

[80] K. U. Lefevre and B. Tollens, *Ber.*, **40**, 4513 (1907)

[81] See: R. L. Whistler, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **24**, 13 (1940)

reaction, when the decarboxylation is conducted under mild conditions such that any added pentose could be recovered.[82] Also, in the case of arabinose, the action of boiling 12% hydrochloric acid causes a 70 to 80% conversion to furfural, but in the case of galacturonic acid only 42% furfural is obtained.

2-Keto and 5-keto aldonic acids also give carbon dioxide and furfural (see below) in yields similar to those for the uronic acids. However, ascorbic acid, as discussed later, gives a very high yield (above 80%) of furfural. "Reductic acid", an enolic substance similar in structure to the

$$CH_2 \quad CH_2 \quad C=C \quad -C=O$$
$$\qquad\qquad\qquad | \quad |$$
$$\qquad\qquad\quad OH \; OH$$
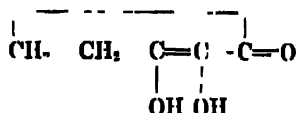
ascorbic acids, has been isolated[83] by the action of strong acid on pentoses and uronic acids. It is conceivable that decarboxylation and furfural formation proceed through an enolic intermediate of this type. The conversion of 2-keto acids to the ascorbic acid analogs is always accompanied by some furfural formation.

The aldobiuronic acids liberate carbon dioxide and form furfural in a manner similar to the uronic acids. With polysaccharide materials the formation of carbon dioxide is considered very strong evidence for the presence of uronic acids. The evidence for a biological formation of pentosan material by the decarboxylation of uronic acid groupings is very weak, however, for some polyuronide materials contain both arabinofuranose and galactopyranose units (see Chapter XV).

The presence of both aldehydic and acidic groups in uronic acids allows the formation of numerous types of derivatives. Phenylhydrazine will form hydrazides, hydrazones and osazones. The action of acidic methanol leads to the formation of the ester of the glycuronide. Rate studies have shown that the esterification reaction is 25 to 55 times as fast as glycoside formation, in the case of galacturonic acid.[84] Reaction for 66 hours at 0° gave a good yield of the pure ester of methyl glucuronide. If the unesterified glycoside is desired, the ester grouping can be hydrolyzed with alkali and either the uronide or the uronide salt prepared.

All three natural uronic acids have been isolated as the free acids in crystalline form. Glucuronic acid is known only as the beta form, whereas the other two exist as alpha- and beta-pyranoid forms. The crystalline γ-lac-

[82] C. M. Conrad, J. Am. Chem. Soc., 53, 2282 (1931).
[83] T. Reichstein and R. Oppenauer, Helv. Chim. Acta, 16, 988 (1933), 17, 390 (1934)
[84] E. F. Jansen and R. Jang, J. Am. Chem. Soc., 68, 1475 (1946).

tones of glucuronic and mannuronic acid have been prepared, and are
known as glucurone and mannurone.

β-Glucopyruronic   Glucurone
acid   (pyranoid ring
structure uncertain)
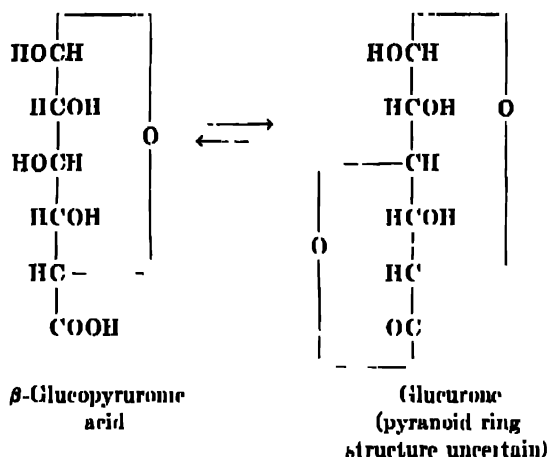
The action of acidic methanol on glucurone leads to the formation of
pyranoside and furanoside derivatives.[*] In the cold, the methyl fururono-
side γ-lactone is formed. This product can be changed by the action of hot
methanol-HCl to the pyruronoside methyl ester. It is interesting that the
fururonoside lactone reduces Fehling solution and has an abnormal ab-
sorption curve in alkali. These reactions have been attributed to the pres-
ence of two five-membered rings, one of which splits with the formation of
enols as has been noted for the dilactones of saccharic acids (see p. 302).

D. Keto Aldonic Acids. The keto aldonic acids of the hexose series are of
the 2- and 5-keto types. The 2-keto acids have been called osonic acids be-
cause of their preparation by the oxidation of osones. The 5-keto acids have
been termed keturonic acids, uronic acids related to ketoses, whereas the

2-Ketogluconic acid   5-Ketogluconic acid
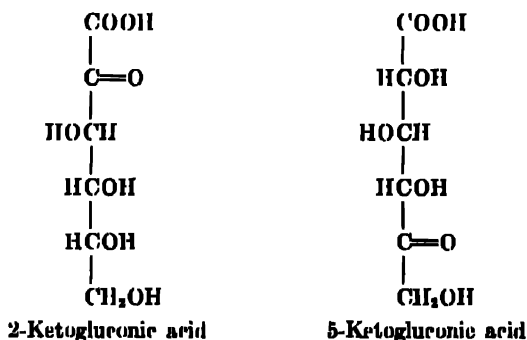
[*] L. N. Owen, S Peat and W. J. G Jones, *J. Chem. Soc.*, 339 (1941).

normal uronic acids are alduronic acids. Both types of keto acids show a great similarity to uronic acids in their color reactions and in the property of decarboxylation on heating with acids. The 2-keto acids, however, show a distinct difference in their ready enolization to ascorbic acid analogs (see page 314). In this discussion, the term uronic acid will be reserved for the alduronic acids.

2-Ketogluconic acid has been isolated from a polysaccharide occurring in Irish moss,[86] but no other similar product has been found in nature. Both 2-keto- and 5-keto-gluconic acids have been prepared by the action of bacteria.

A number of methods are available for the synthesis of 2-keto acids. Gluconic acid methyl ester can be oxidized[87] with $NaClO_3$ and V_2O_5 (see also under Halic acid oxidations). Similar reactions produce the corresponding 2-keto acids of galactose, glucoheptose and galaheptose. Galactosazone is oxidized by bromine to 2-ketogalactonic (or galactosonic) acid.[88] Similarly, maltosazone is converted to the 2-ketomaltobionic acid. "Betadiacetonefructose" is oxidized by potassium permanganate to diisopropylidine-2-ketogluconic acid.[89] Careful oxidation of unsubstituted ketoses with nitric acid has been partially successful. Bacterial action on glucose has given[90] yields as high as 80% of the 2-keto acid (see later in this chapter). Finally, direct synthesis of 2-keto-L-erythronic acid from 2-hydroxy-3-butenenitrile in a series of steps has been reported.[91]

The 5-keto acids have been prepared by three general methods. Bacterial oxidation of glucose gives a 90% yield of 5-ketogluconic acid.[92] 5-Keto-L-galactonic acid has been formed from D-galacturonic acid by the action of calcium and strontium hydroxides,[93] but barium hydroxide gives different strongly reducing products. Glucuronic acid appears to behave similarly.

The permanganate oxidation of diisopropylideneglucatose leads to the formation of 5-ketogalactonic acid.[94]

The keto acids show some similarity to ketoses in their behavior toward oxidizing agents.[95] 5-Ketogalactonic acid is not affected by bromine water at 15 20°. It reacts with sodium hypoiodite but only one atom of iodine is

[86] E. G. Young and F. A H. Rice, J Biol. Chem , 164, 35 (1946).
[87] P. P. Regna and B. P Caldwell, J Am Chem Soc , 66, 243 (1944).
[b] T. Kitasato, Biochem. Z , 207, 217 (1929).
[89] H. Ohle and R. Wolter, Ber., 63, 843 (1930).
[90] J. J. Stubbs, L. B. Lockwood, E. I Roe, B. Tabenkin and G. E. Ward, Ind. Eng. Chem., 32, 1626 (1940).
[91] A. Th. Kuchlin, Rec. trav. chim , 49, 705 (1930).
[92] L. B. Lockwood, B. Tabenkin and G. E. Ward, J. Bact., 49, 51 (1941).
[93] F. Ehrlich and R. Guttmann, Ber., 67, 573 (1934).
[94] T. Reichstein and W. Bosshard, Helv. Chim. Acta, 17, 753 (1934)
[95] H. Ohle, Ber., 67, 155 (1934).

consumed; 2-ketogluconic acid does not react with this agent in the cold. Highly purified salts of the acid reduce Fehling solution in the cold very slowly. A modified Benedict solution reacts readily with 5-ketogluconic acid; complete oxidation occurs at 25° in 7 to 14 minutes, whereas 2-ketogluconic acid, glucose, fructose, uronic acids and simple aldehydes do not react appreciably under these conditions.[96] Hence, a quantitative estimation is possible in the presence of these latter materials. Quantitative estimation of 2- and 5-keto acids has been carried out[90] by the Shaffer-Hartmann method; 2-ketogluconic acid has 87% of the reducing power of glucose and 5-ketogluconic acid, 80%.

The similarity of the keto aldonic acids and uronic acids has been mentioned earlier. 2-Ketogluconic acid gives a 33% yield of furfural in four hours and the 5-keto acid 12.5% , when heated with 12% hydrochloric acid.[97] The evolution of carbon dioxide from the 5-keto acid is quantitative. 2-Keto-L-arabonic acid, prepared from the osone, loses carbon dioxide similarly, but the final product is not furfural but L-erythrose, isolated as the phenylosazone or as calcium L-erythronate after bromine oxidation.[98]

The well-known naphthoresorcinol color is slowly developed by 2-ketogluconic acid in a manner resembling galacturonic acid.

2-Ketogluconic acid and its lactone exist only as sirups although hygroscopic crystals of the former have been reported.[91] Esters can be easily prepared by the action of methanol and sulfuric acid on the sodium salt.[99, 99] The ester and salt mutarotate in the same direction as fructose. Ultraviolet absorption spectra[100] of solutions of the salts and acid indicate the absence of carbonyl or carboxyl groups, and, for alkaline solutions, the absorption is typical of an ethylenic or enolic linkage.

The keto aldonic acids have been investigated primarily as intermediates in the synthesis of ascorbic acids; 2-keto-L-gulonic acid is the most important of this series. The degradation of these acids to simpler acids has been utilized. Thus, 5-keto-D-gluconic acid can be oxidized by oxygen in alkaline solution with various catalysts or by nitric acid to tartaric and oxalic acids.[101, 102]

5-Ketogluconic acid in sirupy form is unstable, turns black in a short time and froths with the liberation of gas.[102]

[96] W. E. Militzer, *J. Biol. Chem.*, *154*, 325 (1944).

[97] E. G. Young and F. A. H. Rice, *J. Biol. Chem.*, *64*, 35 (1946); F. Ehrlich and R. Guttmann, *Ber.*, *67*, 573 (1934).

[98] A. M. Gakhokidze, *J. Gen. Chem., U.S.S.R.*, *11*, 109 (1941).

[99] H. Ohle and G. Berend, *Ber.*, *60*, 1159 (1927).

[100] P. Niederhoff, *Z. physiol. Chem.*, *181*, 83 (1929).

[101] R. Pasternack and P. P. Regna, U. S. Patent 2,203,923, June 11, 1940.

[102] W. E. Barch, *J Am. Chem. Soc.*, *55*, 3653 (1933)

E. Ascorbic Acids.[101]

a. GENERAL PROPERTIES AND REACTIONS

Ascorbic acids, of which the best known is Vitamin C or L-xyloascorbic acid, may be considered as reductones (I) or as represented by the general

$$R—C=C—C—R' \qquad (I)$$
$$\overset{|}{HO}\ \overset{|}{OH}$$

(with O double-bonded at the carbonyl position)

formulas II and III. The compounds are characterized by an *enediolic* system. Varying degrees of antiscorbutic activity are shown by compounds of this group, but only the compounds of type II, with the lactone ring on the right, are active in this respect.

(II)   (III)
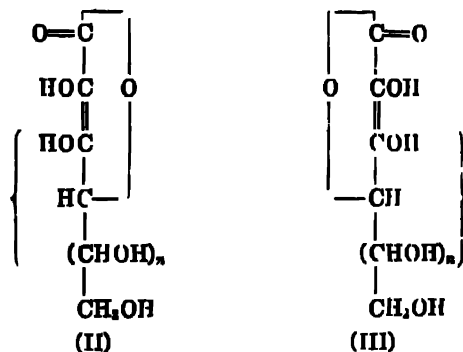
The nomenclature of the ascorbic acids is based on the configuration of the osone actually or hypothetically used in its preparation (see below), the portion concerned is that shown in the bracket.[104] Since carbon atom

(IV)   (V)
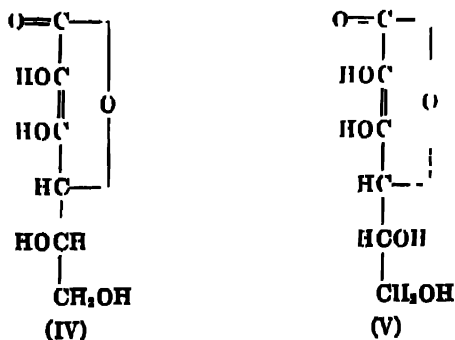
[103] F. Smith, *Advances in Carbohydrate Chem*, *2*, 79 (1946)

[104] A better nomenclature might be to use the configuration of the asymmetric carbons actually present. Compound IV would be L-*threo*-ascorbic acid and V, D-*erythro*-ascorbic acid.

three is not asymmetric, dual names are possible. For Vitamin C, the names are L-xylo- or L-lyxo-ascorbic acid (IV). Formula V represents D-arabo-ascorbic acid, which has $\frac{1}{20}$ the antiscorbutic activity of IV.

The ascorbic acids can be considered as enolic lactones of the 2-keto and 3-keto aldonic acids. For Vitamin C, there apparently exists an equilibrium between the ascorbic acid and the 3-keto acid. The latter has not been isolated, but hydrazone and "osazone" derivatives have been prepared.[105]

(VI)   (VII)   (VIII)
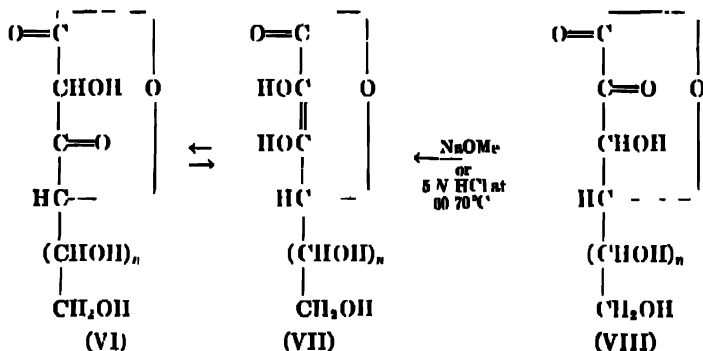
Under normal conditions, the 2-keto acid apparently does not participate in the equilibrium. The kinetics of conversion of 2-keto acids to the ascorbic acids has been studied.[106] The yields ranged from 70% for the 2-ketogulonic acid system to only 6% for 2-keto-D-galactoheptonic acid.

Four general methods are available for the preparation of ascorbic acids. The two most applicable involve the enolization of keto acids; the others involve condensations.

Enolization and Lactonization of 2-Keto Aldonic Acids.[107] By the action of sodium methylate on the methyl esters, 2-keto acids are transformed into ascorbic acids (see VII and VIII). The reaction is almost quantitative. Lactonization and enolization take place simultaneously. Heat treatment of an aqueous solution of the free acid causes only a limited amount of conversion. Acids also act as catalysts (see above). From the acid hydrolyzate of the methyl glycoside of 3,4-isopropylidene-2-keto-L-ribonic acid, the 2-keto-L-ribonic acid could not be isolated, because L-erythroascorbic acid was formed very rapidly.[108]

Cyanohydrin Synthesis from Osones.[109] For this method, 3-keto aldonic

[105] E. G. Cox, E. L. Hirst and R. J. W. Reynolds, *Nature*, *130*, 888 (1932).

[106] P. P. Regna and B. P. Caldwell, *J. Am. Chem. Soc.*, *66*, 246 (1944).

[107] K. Maurer and B. Schiedt, *Ber.*, *66*, 1054 (1933).

[108] T. Reichstein, *Helv. Chim. Acta*, *17*, 1003 (1934).

[109] T. Reichstein, A. Grussner and R. Oppenauer, *Helv. Chim. Acta*, *16*, 561 (1933); R. G. Ault, D. K. Baird, H. C. Carrington, W. N. Haworth, R. W. Herbert, E. L. Hirst, E. G. V. Percival, F. Smith and M. Stacey, *J. Chem. Soc.*, 1419 (1933).

acids are formed as intermediates which are not isolated. The first product, the nitrile, immediately enolizes with simultaneous ring formation to an *imino* analog (XI) of the ascorbic acid, and the latter is formed by removal

```
HCN            CN           HN=C—         O=C—
 +             |            |    |        |   |
HC=O          CHOH         HOC  |         HOC |
 |      →      |     →      ‖    | O   →   ‖   | O
C=O           C=O          HOC  |         HOC |
 |             |            |    |        |   |
CHOH          CHOH         HC—             HC—
 |             |            |              |
(IX)          (X)          (XI)           (XII)
```
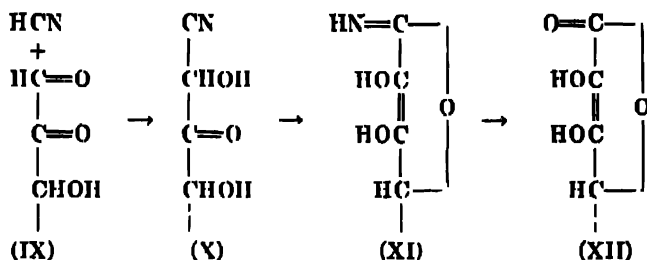
of the imino group with dilute acid. The osones must be in a very pure state in order to insure a good yield of final product. The customary configurational names of ascorbic acids are based on this method.

Condensation of Hydroxy Aldehydes with Ethyl Glyoxalate or Mesoxalate.[110] The intermediate 3-keto ester is not isolated. D-Glucoheptoascorbic acid was prepared in this way from glucose.

```
COOEt         ⌈ COOEt ⌉      O=C—
 |            |        |     |   |
CHO           | CHOH   |     HOC |
 |      →     |        |  →  ‖   | O
CHO           | C=O    |     HOC |
 |            |        |     |   |
CHOH          | CHOH   |     HC—
              ⌊        ⌋     |
```
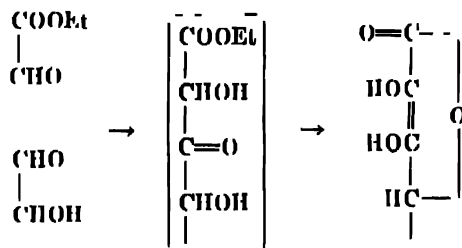
Condensation of Esters of Hydroxy Acids.[111] This method is similar to the Claisen condensation

```
COOEt                            O=C—
 |                               |   |
CHOB/         CH₂OB/            HOC  O
 |       +     |        NaOEt   ‖    |
(CHOH)ₙ        COOEt     →      HOC  |
 |                               |   |
CH₂OH                           HC—
                                 |
                               (CHOH)ₙ
                                 |
                               CH₂OH
```
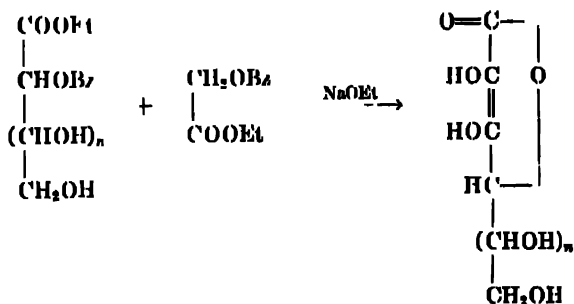
[110] B. Helferich and O. Peters, *Ber.*, **70**, 465 (1937).
[111] F. Micheel and H. Haarkoff, *Ann.*, **545**, 28 (1940).

The ascorbic acids are weak acids as a result of the presence of the enolic groups rather than of the lactone ring.[106, 112] They reduce Fehling solution, and the double bond is oxidized by acidic iodine solution. The reaction with iodine is used as a quantitative method to distinguish them from 2-keto acids. The action of boiling 12% HCl causes the formation of furfural in very high yields, above 80% (see above).

b. Vitamin C (L-Xyloascorbic Acid)[113]

Vitamin C is widely distributed in nature, especially in green vegetables and citrus fruits. It has been found in conifer needles, and its presence in the lowly potato provides an excellent dietary source for those unable to secure other foods. It is universally distributed in plant tissues, normally in the reduced form. When the tissues are damaged, the ascorbic acid is oxidized as a result of various causes, including the presence of a specific ascorbic oxidase. The equilibrium between the ascorbic acid (XIII) and the oxidation product, dehydroascorbic acid, (XIV) is very important to plant and animal life.[114] The ascorbic acid apparently functions as a hydrogen carrier. In rabbit liver 27% of the total acid has been found in the reduced form and in muscle tissue, 50%. Fresh human milk contains only ascorbic acid.

$$
\begin{array}{ccc}
\text{O=C---} & \text{O=C---} & \text{COOH} \\
\text{HOC} \quad\ \ \text{O} & \text{O=C} \quad\ \ \text{O} & \text{C=O} \\
\text{HOC} & \text{O=C} & \text{C=O} \\
\text{HC---} & \text{HC---} & \text{HCOH} \\
\text{HOCH} & \text{HOCH} & \text{HOCH} \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH} \\
(\text{XIII}) & (\text{XIV}) & (\text{XV})
\end{array}
$$
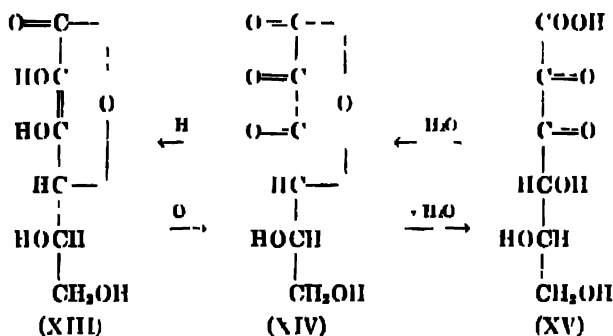
The 2,3-diketo-L-gulonic acid (XV) is formed spontaneously on dissolution of dehydroascorbic acid (XIV).[115] In contrast, ascorbic acid is a weakly acidic substance and has little tendency to hydrolyze.

Ascorbic acid is very sensitive to oxygen, especially in alkaline solution, and to acidic iodine. The enolic grouping can be split by hypoiodite to form oxalic and L-threonic acids.[116] Methylation with diazomethane at 0° forms

[112] R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds and F. Smith, *J. Chem. Soc*, 1270 (1933).

[113] H. R. Rosenberg, "Chemistry and Physiology of the Vitamins," p. 289, Interscience Publishers, New York (1942).

[114] W. O. James and J. M. Cragg, *New Phytologist*, 42, 28 (1943).

[115] J. R. Penney and S. S. Zilva, *Biochem. J.*, 39, 1 (1945).

[116] E. L. Hirst, *J. Soc. Chem. Ind.*, 52, 221 (1933).

a 3-methyl ether, which no longer reduces, but which is still acidic.[117] Methylation at 20° forms a 2,3-dimethyl ether which does not react with hydrazines.

The stability of ascorbic acid in plant products is very important in the food industry. Oxidation in milk is accelerated by copper and sunlight. Low temperature storage of foods (below 42° *F.*) is helpful in preventing loss.

In 1928, a strongly reducing acid termed a "hexuronic acid," was isolated[118] from oranges and cabbages, which was later shown to be identical with vitamin C, isolated earlier in an almost pure state.[119] The constitution[120] was established in 1933 and, the first successful synthesis was described in the same year.[109]

The first synthesis was based on the addition of HCN to L-xylosone. D-Galacturonic acid was the starting material; reduction gave L-galactonic acid, and the amide was then degraded to L-lyxose, which was converted to L-xylosone. The most important commercial method utilizes sorbitol as the starting material.[121] Bacterial oxidation produces L-sorbose, and the diacetone derivative (I) is oxidized with permanganate to diisopropylidene-2-keto-L-gulonic acid (II) which after hydrolysis of the acetone groups (III) can be converted to ascorbic acid (IV).

(Ip = (CH)₂C —)
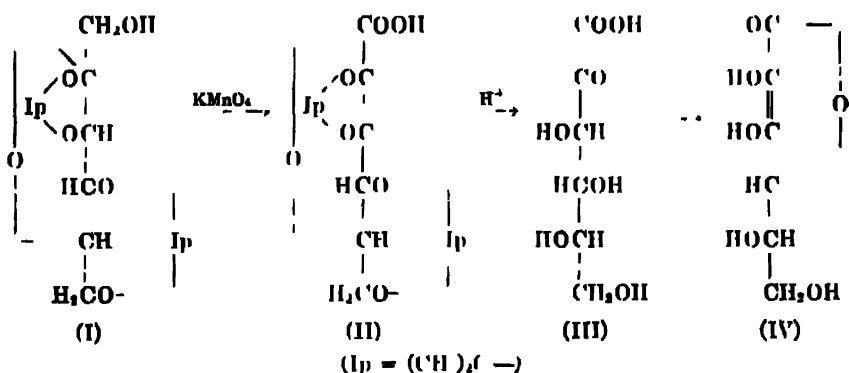
The conversion of D-galacturonic acid to L-galactonic acid, and subsequent oxidation to 2-keto-L-galactonic acid has been suggested as a method.[121] Sorbose can be oxidized directly to the 2-keto-L-gulonic acid, but better yields are obtained with the diacetone derivatives

[117] T. Reichstein and R Oppenauer, *Helv. Chim Acta*, 17, 390 (1934)

[118] A. Szent-Györgyi, *Biochem. J.*, 23, 1387 (1928)

[119] S. S. Zilva, *Biochem. J.*, 21, 689 (1927).

[120] R. W. Herbert, E. G. V. Percival, R. J. W Reynolds, F. Smith and E. L. Hirst, *J. Soc. Chem. Ind*, 52, 221, 482 (1933), F. Micheel and K Kraft, *Z physiol Chem*, 222, 235 (1933)

[121] T. Reichstein and A. Grüssner, *Helv. Chim Acta*, 17, 311 (1934)

[122] H. S Isbell, *J. Research Natl. Bur. Standards*, 33, 45 (1944).   P. P. Regna and

F. Osones. The osones are known primarily in the form of their hydrazine derivatives, the osazones. These "dicarbonyl" sugars have recently achieved a great amount of importance as intermediates in the synthesis of ascorbic acids. They also can be oxidized to 2-keto aldonic acids.

The osones exist only as amorphous or sirupy materials. They are very labile and show the characteristics of enediols of the reductone type. For this reason the formula for D-glucosone, for example, might be represented best by (VI) below, rather than (V). This formula is similar to a "reduced"
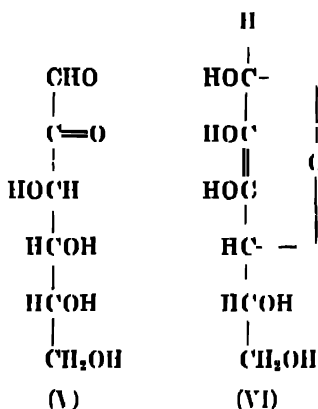
ascorbic acid. Reduction occurs with Fehling solution in the cold, and derivatives are obtained with hydrazines and diamines.

Several methods are available for the preparation of these compounds. Osazones can be hydrolyzed by acids or by carbonyl compounds. (See under Osazones.) Alcohol-insoluble osazones are generally hydrolyzed with concentrated hydrochloric acid at a low temperature. Alcohol-soluble osazones can be split by the action of benzaldehyde. Pyruvic acid has also been used.

Catalytic oxidation of sugars and alcohols is a more direct method. Hydrogen peroxide and iron salts were used originally (see under hydrogen peroxide oxidations). However, much better yields have been obtained by the direct oxidation with cupric salts.[121] The action of a limited excess of cupric acetate for a short time on methanol solutions of L-sorbose or L-xylose has given a 60% yield of the osone.

The simplest osone, glycerosone or hydroxypyruvic aldehyde, has been prepared by the oxidation of dihydroxyacetone. This compound is enolic in

B. P. Caldwell, *J. Am. Chem. Soc.*, 66, 243 (1944); R. Pasternack and P. P. Regna, U. S. Patent 2,207,991, July 16, 1940; 2,338,534, Jan. 4, 1944.

[121] R. Weidenhagen, *Z. Wirtschaftsgruppe Zuckerind.*, 87, 711 (1937).

character, reducing cold Fehling solution and forming acidic aqueous solutions.[124] It exists normally as the trimer.

Glucose has been oxidized with *A. parasiticus* Speare and another unidentified mold.[125] Yields of 8.6% of glucosone were obtained from glucose and 17% from maltose. Starch and sucrose gave 15 and 13.6% yields, respectively.

Substituted osones can be synthesized by the Grignard reaction. Diisopropylidene-2-ketogluconic acid and phenyl magnesium bromide react to form 1-C'-phenyl-2,3,4,5-diisopropylidene-glucosone.[126] Some 1,1-C'-diphenyl-2,3,4,5-diisopropylidenefructose is also formed. Hydrolysis with boiling normal sulfuric acid in propanol forms the 1-C'-phenyl-glucosone. This product is the first osone prepared in crystalline form.

A tetraacetylglucosone hydrate is prepared by the treatment of tetraacetyl-1,2-glucoseen with chlorine followed by silver carbonate (see under Glycoseens).

2. Oxidation Agents

A. Halogen Oxidations.[127] The halogens and their oxyacids probably are the most important oxidants used in the carbohydrate field. They are widely used as bleaching agents, but the mechanism of this action remains to be clarified. As reagents for preparatory purposes (particularly for aldonic acids and lactones) and for analytical procedures, they are very important. Periodic acid, discussed in a later section, has an important application for the elucidation of structures of carbohydrates. A number of valuable commercial products are made by treatment of polysaccharides with halogens, particularly chlorine or hypochlorous acid, but the nature of these actions, such as the modification of starch, has not been clarified.

Bromine and hypoiodite oxidations are particularly suitable for the preparation of aldonic acids from aldoses. Similarly, uronic acids are converted to saccharic acids. Of less value is the oxidation of primary alcoholic to aldehydic groups. In this manner, glycosides can be converted to uronides and polyols to aldoses and aldonic acids.

Secondary alcoholic groups are oxidized to keto groups, and the 2-keto and 5-keto acids are formed in this manner. More extended oxidation results in the cleavage of carbon-carbon bonds and the production of short chain acids.

[124] W. E. Evans, Jr., C. J. Carr and J. C. Krantz, Jr., *J. Am. Chem. Soc.*, *60*, 1628 (1938); R. G. W. Norrish and J. G. A. Griffiths, *J. Chem. Soc.*, 2829 (1928).

[125] C. R. Bond, E. C. Knight and T. K. Walker, *Biochem. J.*, *31*, 1033 (1937).

[126] H. Ohle and I. Blell, *Ann.*, *492*, 1 (1931).

[127] J. W. Green, *Advances in Carbohydrate Chem.*, *3*, 129 (1947).

Periodic acid is of great value in that it usually produces quantitative cleavage of pairs of vicinal hydroxyl groups and the formation of dialdehydes. Oxidations of this type are discussed in the next section.

It is particularly interesting that in spite of the cheapness and availability, chlorine and hypochlorite are not common oxidation agents in this field.

a. HALOGENS AND HYPOHALITES

The use of halogens and hypochlorites as oxidizing agents is complicated by the change in the nature of the oxidation as the conditions of temperature, acidity and concentration vary. The halogens not only show considerable difference in the position of the various equilibria and the speed at which the equilibria are attained, but also in the maximal concentrations as expressed by the solubilities.

At 20° C. the solubility[128] of the halogens in water is: chlorine, 1.85 g./100 ml.; bromine, 3.58 g./100 ml.; and iodine, 0.28 g./100 ml. In aqueous solution, hydrolysis occurs as expressed by the following equation:

$$X_2 + H_2O \leftrightarrow HOX + HX$$

The equilibrium constants for the reaction are given[129] as:

Chlorine, $K = 4.5 \times 10^{-4}$
Bromine, $K = 2.4 \times 10^{-8}$
Iodine, $K = 3.6 \times 10^{-13}$

Evidently in acid solution, the equilibrium lies far to the left and the concentration of hypohalous acid is very small.

When alkali is added to the system, the concentration of hypohalite ion increases:

$$X_2 + 2NaOH \rightleftarrows NaOX + NaX + H_2O$$

Hence, the concentration of free halogen, halic acid and hypohalite will vary greatly with the acidity. For 0.02 M chlorine solutions at room temperature, for example, Ridge and Little[130] have shown that at pH 1, 82% of the total chlorine exists as free chlorine and 18% as hypochlorous acid. At pH 4, only 0.4% is free chlorine and 99.6% is hypochlorous acid. At pH 8, 21% exists as hypochlorous acid and 79% as hypochlorite. Obviously,

[128] A. Seidell, "Solubilities of Inorganic and Metal Organic Compounds," Vol. 1, Van Nostrand, New York (1940).

[129] J. W. Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. 2, Longmans, Green and Co., London (1927).

[130] B. P. Ridge and A. H. Little, J. Textile Inst., 33, T33 (1942).

the concentration of the oxidant and probably the nature of the oxidation will be influenced greatly by the acidity.

Hypohalites are converted to halates according to the equation:

$$2 HOX + OX^- \rightleftarrows 2 HX + XO_3^-$$

For hypochlorous acid,[141] the minimum stability exists at pH 6.7 and the maximum stability at pH 13. Various anions exert a catalytic effect. For hypobromite solutions, these positions of maximum and minimum stability are shifted to more alkaline conditions. The velocity of halate formation increases greatly in the order: $ClO_3 < BrO_3 < IO_3$.

The above discussion should emphasize the difficulties involved in determining the nature of the active oxidant in systems containing halogens or hypohalites and in showing that marked effects may be observed as a result of slight variations in the conditions of the oxidations.

Oxidation in Acid Solutions. In acid solutions the active oxidant is the free halogen or the hypohalous acid. As noted above, the proportions of these potential forms of the oxidant vary with the acidity of the solution and the nature of the halogen. However, unless a buffer or neutralizing substance is present, the solution will become strongly acid as a result of the formation of hydrohalic acid

$$RCHO + Br_2 + H_2O \rightarrow RCOOH + 2HBr$$
$$RCHO + HOBr \rightarrow RCOOH + HBr$$
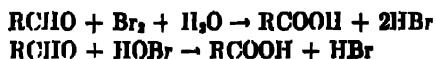
Hlasiwetz[142] first used halogens for the oxidation of sugars. Lactose was treated with bromine and glucose with chlorine. Gluconic acid was formed from glucose and isolated as the calcium salt. Kiliani[143] found that sugars were oxidized readily by bromine at room temperature and obtained yields of 50 to 70% of various aldonic acids.

The accumulation of HBr during the oxidation produces a definite inhibition of the rate of oxidation. The effect is more than one of an increasing acidity, for, although other strong acids also inhibit the rate, the effect is largest for HBr and HCl.[144] To minimize this inhibiting influence, the reaction may be carried out in the presence of a buffer such as barium carbonate or barium benzoate.[145] In general, the presence of buffers increases

[141] R. M. Chapin, *J. Am. Chem. Soc.*, **56**, 2211 (1934)

[142] H. Hlasiwetz, *Ann. 119*, 281 (1861), H. Hlasiwetz and J. Habermann, *ibid.*, **155**, 120 (1870).

[143] H. Kiliani and S. Kleeman, *Ber*, *17*. 1296 (1884).

[144] H. H. Bunzel and A. P. Mathews, *J. Am. Chem. Soc.*, **31**, 464 (1909).

[145] H. A. Clowes and B. Tollens, *Ann.*, *310*, 164 (1899); C. S. Hudson and H. S. Isbell, *J Am. Chem. Soc.*, **51**, 2225 (1929); *J. Research Natl. Bur. Standards*, **3**, 57 (1929).

the yields of aldonic acids, and, in addition, hydrolysis of disaccharides is prevented. Yields of 96% of gluconic acid and of 90% of xylonic acid (as salts) have been obtained when buffered solutions were employed.

When the oxidation period is extended, particularly under unbuffered conditions, keto acids may be formed in small yields. Rhamnose gives 5-ketorhamnonic lactone[136] and hexose sugars the 5-keto acids.[137] Under more drastic conditions, carbon-carbon bonds are cleaved with the production of short-chain acids.

A variation of the bromine oxidation process which seems to be particularly feasible for the commercial production of aldonic acids involves the electrolysis between carbon electrodes of solutions containing sugars, small amounts of bromides, and a buffer such as calcium carbonate.[138] Presumably the reaction takes place by the formation of free bromine at the anode; the bromine oxidizes the aldose to the aldonic acid and is reduced to bromide. Yields are almost theoretical in many cases. If the electrolytic method is not well controlled, saccharic acids and 2-keto and 5-keto aldonic acids may be produced.[139]

The ketoses are resistant to the action of bromine,[140] bromine oxidation is used sometimes to remove aldoses from mixtures such as invert sugar. By extending the period of oxidation and employing high temperatures, Kiliani obtained oxalic acid, bromoform and glycolic acid.[141] Milder conditions give keto acids such as 5-keto-L-gulonic acid from fructose and 5-keto-L-gluconic acid from sorbose.[142]

For polyols, more drastic oxidative conditions are required than for aldoses. The oxidation product of sorbitol gives two osazones, glucosazone and gulosazone.[143]

The mechanism of the oxidation of aldoses by bromine in the presence of barium carbonate and bromides (pH about 5.4) has been studied by Isbell and Pigman.[144] Under these conditions the active oxidant is free bromine and not hypobromous acid.

It is interesting that the ring forms of the sugars rather than the free

[136] E. Votoček and S. Malachata, Anales soc. espan fis y quim., 27, 494 (1929)

[137] J. P. Hart and M. R. Everett, J. Am. Chem Soc., 61, 1822 (1939)

[138] H. S. Isbell and H. L. Frush, J. Research Natl. Bur. Standards, 6, 1145 (1931); H. S. Isbell, U S Patent 1,976,731 Oct 16, 1934, E L Helwig, U. S. Patent1, 895,114 Jan. 21, 1933.

[139] R. Pasternack and P. P. Regna, U S Patent 2,222,155 Nov. 10, 1940; E. W. Cook and R. T. Major, J. Am. Chem Soc., 57, 773 (1935).

[140] H. Kiliani and C. Scheibler, Ber., 21, 3276 (1888)

[141] H. Kiliani, Ann., 205, 182 (1880).

[142] M. R. Everett and F. Sheppard, "Oxidation of Carbohydrates; Keturonic Acids; Salt Catalysis," Univ. of Oklahoma Medical School (1944)

[143] C. Vincent and Delachanal, Compt. rend., 111, 51 (1890); E. Fischer, Ber., 23, 3684 (1890); H W Talen, Rec. trav. chim., 44, 891 (1925).

[144] H. S Isbell and W. W. Pigman, J. Research Natl. Bur. Standards, 10, 337 (1933)

aldehyde are oxidized directly under these conditions.[145] Pyranoses yield δ-lactones and furanoses γ-lactones, directly.

$$
\begin{array}{ccc}
\text{HCOH} & & \text{CO} \\
| & & | \\
\text{HCOH} & & \text{HCOH} \\
| & & | \\
\text{HOCH} \quad O & \xrightarrow{\text{Br}_2} & \text{HOCH} \quad O \quad + 2HBr \\
| & & | \\
\text{HCOH} & & \text{HCOH} \\
| & & | \\
\text{HC} - & & \text{HC} - \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH}
\end{array}
$$

Glucopyranose   Gluconic δ-lactone
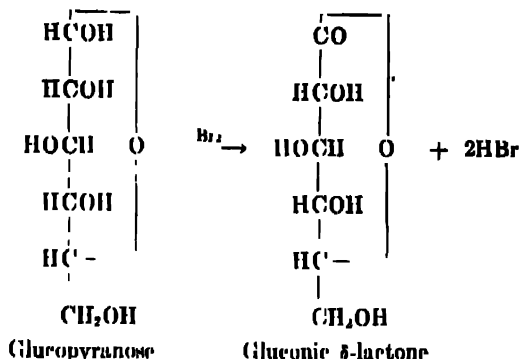
The yields are high. The direct formation of δ-lactones from the sugars provides strong evidence that the crystalline sugars, in general, have pyranoid structures (see Chapter II).

In the hexose series as far as studied, the α-isomers are oxidized much more slowly than the β-isomers.[146] β-Glucose, for example, oxidizes about thirty-five times more rapidly than the α-isomer. The anomeric forms of galactose show a similar difference as shown in Fig. 2. The data for a number of sugars are given in Table IV. When plotted on a semilogarithmic scale, the rate curves for the oxidation are approximately linear. Fig. 3 shows the data for several forms of mannose.

The equilibrium solutions are oxidized at rates intermediate between those for the individual anomers (see Figs. 2 and 3), and the oxidation curve is composed of a rapid phase followed by a slow phase. Extrapolation of the slow portion (on a semilogarithmic plot) to zero time gives the amount of the two anomers in the equilibrium solution. The composition of equilibrium solutions of several sugars as determined in this manner agrees with that obtained by optical rotation studies (see Table III, Chapter II).

One form of mannose (mannose·CaCl₂·4 H₂O, Fig. 3) exhibits an oxidation curve intermediate between those for the α- and β-forms, and considerable mannonic γ-lactone is present in the solution. Consequently, it would appear that this modification is a mannofuranose.

Oxidation with Hypohalites in Alkaline Solutions. In alkaline solution the halogens exist as hypohalous acid and hypohalite ions. The oxidation is likely to be more drastic than for the free halogens. Thus, whereas free

[145] H. S. Isbell, *J. Research Natl. Bur. Standards*, 8, 615 (1932); H. S. Isbell and C. S. Hudson, *ibid.*, 8, 327 (1932).
[146] H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, 18, 141 (1937).

iodine will not act as an oxidant, hypoiodite is a powerful oxidizing agent. Hypobromite and hypochlorite particularly are likely to produce oxidation of primary and secondary alcoholic groups and cause cleavage of carbon-
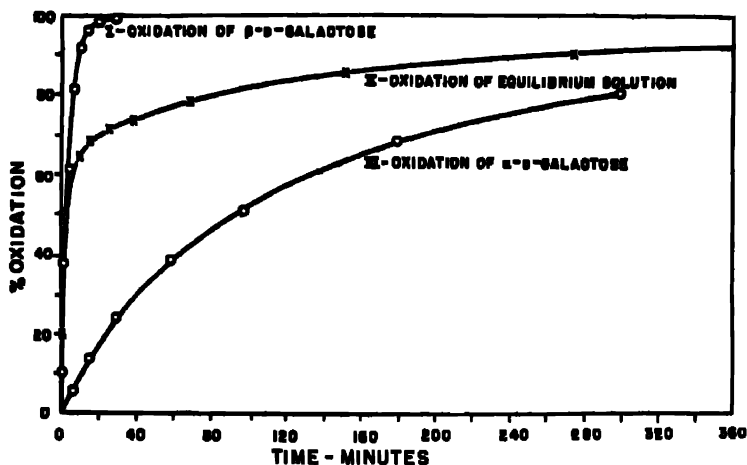
Fig. 2. Rate of oxidation of D-galactose by bromine (ca. 0° C, pH = 5.4, buffered). (After Isbell and Pigman.)
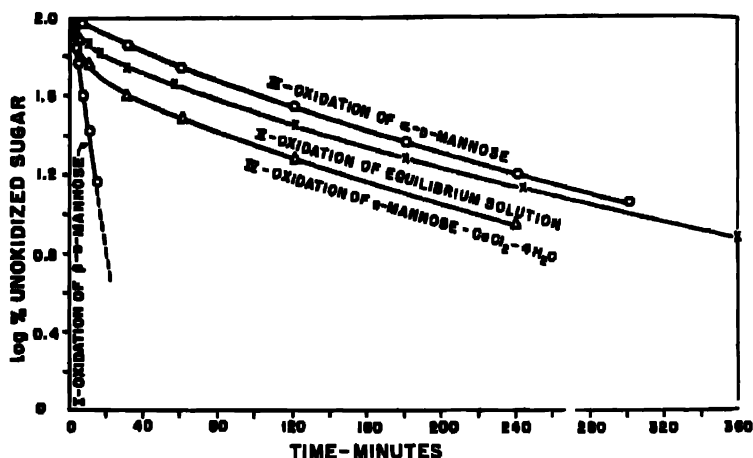
Fig. 3. Rate of oxidation of D-mannose by bromine (ca. 0° C, pH = 5.4, buffered). (After Isbell and Pigman.)

carbon bonds. As noted above, the processes are complicated by the tendency of hypohalite to be converted to halate ions.

Alkaline hypoidite has been proposed as a reagent for the quantitative determination of aldehyde groups.[147] With careful control of conditions,

[147] G. Romijn, Z. anal. Chem., 36, 349 (1897); see also discussion in Chapters III and XII.

TABLE IV

The Rates of Oxidation of the Alpha and Beta Sugars in Aqueous Solutions Containing
0.05 Mole Sugar and 0.08 Mole Free Bromine per Liter and
Buffered with Barium Carbonate and Carbon Dioxide

| Sugar | Oxidation with Bromine Water | | |
	Average value for velocity constant $k \times 10^3$	Relative reaction rates $\dfrac{k_{sugar}}{k_{\alpha\text{-D-glucose}}}$	Ratio of the rates for the α and β isomers k_β/k_α
α-D-Glucose	32	1	39.2
β-D-Glucose	1255	39	
α-D-Mannose	51	1.6	15.3
β-D-Mannose	781	24	
α-D-Galactose	42	1.3	37.9
β-D-Galactose	1590	50	
α-D-Talose	78	2.4	10.8
β-D-Talose (from equilibrium solution).	844	26	
α-D-Gulose·CaCl$_2$·H$_2$O	71	2.2	5.9
β-D-Gulose (from equilibrium solution).	418	13	
β-L-Arabinose	95	3.0	17.5*
α-L-Arabinose·CaCl$_2$·4H$_2$O	1658	52	
α-D-Xylose	90	2.8	18.6
β-D-Xylose (from equilibrium solution).	1673	52	
α-D-Lyxose	156	4.9	2.9
β-D-Lyxose	440	14	
D-Ribose (crystalline)	196	6.1	5.2
D-Ribose (from equilibrium solution).	1010	32	
L-Ribose (crystalline)	195	6.1	7.5
L-Ribose (from equilibrium solution).	1456	45.5	
α-L-Rhamnose, hydrate	90	2.8	8.6
β-L-Rhamnose (from equilibrium solution).	770	24	
α-Lactose, hydrate	29	0.9	32.8
β-Lactose	952	30	
α-Maltose (from equilibrium solution).	24	0.8	64.0
β-Maltose, hydrate	1528	48	

* k_α/k_β. For the nomenclature difficulties for arabinose, see p. 102.

aldoses are converted practically quantitatively to aldonic acids. Measurement of the iodine consumed gives the amount of aldose originally present.

$$RCHO + I_2 + 3NaOH \rightarrow RCOONa + 2NaI + 2H_2O$$
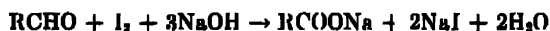
In the reaction, the rate of iodate formation should be slower than the oxidation of the aldose. The reaction is slowed down by the presence of buffers such as borax.[148]

Hypoiodites are used for preparatory as well as analytical purposes. Goebel used barium hypobromite for the preparation of calcium gluconate and maltobionate.[149] In methanol solution, high yields of the aldonic acids are obtained.[150]

Ketoses are essentially inert to the action of hypoiodites under the conditions used for the determination of aldoses, although for accurate work small corrections may be necessary. With excessive amounts of alkali and slightly elevated temperatures, oxalic acid is produced.[151]

More drastic oxidation of aldoses with hypoiodite leads to keto acids and finally to cleavage of carbon-carbon bonds Honig and Tempus[152] claimed to have oxidized glucose stepwise to gluconic acid, 2-ketogluconic acid and D-arabonic acid. However, other workers claim that the main product is 5-ketogluconic acid.[153]

Glycosides are converted by hypoiodite or hypobromite to uronides in rather low yields.[154] Jackson and Hudson[155] obtained a yield of 12% of the

$$
\begin{array}{ccc}
\begin{array}{l} HCOCH_3 \\ | \\ HOCH \\ | \\ HOCH \quad O \\ | \\ HCOH \\ | \\ HC\ -- \\ | \\ CH_2OH \end{array}
&
\xrightarrow{\text{Ba(BrO)}_2}
\begin{array}{l} HCOCH \\ | \\ HOCH \\ | \\ HOCH \quad O \\ | \\ HCOH \\ | \\ HC\ - \\ | \\ COOH \end{array}
\ +\
\begin{array}{l} HCOCH_3 \\ | \\ COOH \\ | \\ \quad O \\ | \\ COOH \\ | \\ HC\ -- \\ | \\ CH_2OH \end{array}
\end{array}
$$
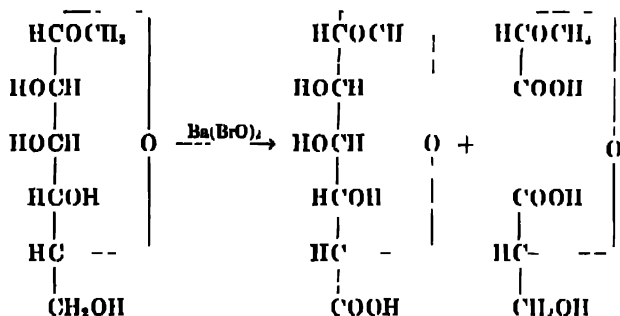
[148] See· K. Myrback and E. Gyllensvard, Svensk Kem Tid , 54, 17 (1942)

[149] W. F. Goebel, J. Biol. Chem , 72, 809 (1927)

[150] S. Moore and K. P. Link, J. Biol Chem., 133, 293 (1940).

[151] K Bailey and R. H Hopkins, Biochem J , 27, 1965 (1933).

[152] M Honig and F. Tempus, Ber., 57, 787 (1924).

[153] T Reichstein and O. Neracher, Helv. Chim Acta, 18, 892 (1935); W. Ruzicka, Z Zuckerind. Böhmen-Mahren, 64, 219 (1941).

[154] M Bergmann and W. W. Wolff, Ber , 56, 1060 (1923); K. Smolenski, Roczniki Chem , 3, 153 (1924)

[155] E. L. Jackson and C. S. Hudson, J Am. Chem Soc., 59, 994 (1937).

brucine salt of methyl α-mannuronide but showed that cleavage of carbon-carbon bonds also occurs.

Polyols are oxidized by alkaline solutions of halogens. Fischer and Tafel[156] obtained 20% yields of glycerosazone by the action of bromine and sodium carbonate on glycerol and subsequent treatment with phenylhydrazine. Galactitol gave an osazone which appeared to be galactosazone. Presumably, the oxidation takes place mainly at the primary alcoholic group.

Amides with free hydroxyl groups at carbon 2 are degraded to sugars with one less carbon atom by treatment with hypochlorites. This is the basis of the Weerman method of degrading sugars, discussed elsewhere (Chapter III).

b. Halic Acids (HXO_3)

Chloric acid in conjunction with catalysts, particularly vanadium pentoxide,[157] has as its principal use the oxidation of aldonic acids or lactones to the 2-keto acids, intermediates in the preparation of ascorbic acid and analogs, as discussed in a preceding section.

D-Gluconic γ-lactone and potassium D-galactonate in methanol solution in the presence of phosphoric acid and V_2O_5 are oxidized by chloric acid to methyl 2-keto D-gluconate and methyl 2-keto-D-galactonate, respectively.[158]

$$
\begin{array}{ccc}
OCOH & & OCOCH_3 \\
| & HClO_3 & | \\
HCOH & \xrightarrow[CH_3OH]{V_2O_5} & C{=}O \\
| & & |
\end{array}
$$
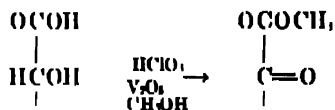
Iodic acid in strong sulfuric acid at 100° C. is reported to show a rather remarkable specificity; ketoses, sucrose and pentoses are oxidized, but aldohexoses and lactose are not attacked.[159] At still higher temperatures, hexoses are oxidized quantitatively to carbon dioxide and water.[160]

Under mild conditions of temperature and in the absence of a catalyst, aldoses, ketoses and sucrose are inert to the action of chloric acid over several weeks time.[161] Bromates in alkaline solution also exert no oxidative action.[162]

[156] E. Fischer and J. Tafel, Ber., 20, 3384 (1887), 22, 106 (1889).

[157] R. Pasternack and P. P. Regna, U. S. Patents 2,203,923 June 11, 1940; 2,207,991 July 16, 1940; 2,188,777 Jan. 30, 1940

[158] P. P. Regna and B. P. Caldwell, J. Am. Chem. Soc., 66, 243 (1944); H. S. Isbell, J. Research Natl. Bur. Standards, 33, 45 (1044)

[159] R. J. Williams and M. Woods, J. Am. Chem. Soc., 59, 1408 (1937)

[160] W. Hurka, Mikrochemie ver. Mikrochim. Acta, 30, 259 (1942).

[161] A. Jeanes and H. S. Isbell, J. Research Natl. Bur. Standards, 27, 125 (1941)

[162] P. Van Fossen and E. Pacsu, Textile Research J., 10, 163 (1946)

c. Chlorous Acid ($HClO_2$)

Chlorous acid is of particular interest because of its use for the removal of lignin and other noncarbohydrates from woody tissue without appreciable action on the carbohydrates. (See Chapter XV, under Holocellulose.) It also is reported to be an effective bleaching agent.

Jeanes and Isbell[161] found that under mild conditions aldoses are oxidized to the aldonic acids but that non-reducing carbohydrates and ketoses are only slowly attacked. The rapidity of oxidation is in the order: pentoses > hexoses > disaccharides; α-hexoses > β-hexoses. The yields of aldonic acids, however, are less than for bromine oxidations.[162] The equation for the oxidation in acidic solution was expressed as:

$$RCHO + 3HClO_2 \rightarrow RCOOH + HCl + 2ClO_2 + H_2O$$

B. Reagents Cleaving Glycols. A number of reagents exhibit relatively sharp specificity for the cleavage of bonds between adjacent carbon atoms carrying hydroxyl groups. The most important of these are periodic acid and lead tetraacetate. The requisite properties of an oxidant of this type have been defined[164] as follows:

1. "The central atom of the oxidant must have a diameter, about 2.5 to 3.0×10^{-8} cm., which is large enough to bridge the space between hydroxyl groups in a 1,2-glycol.

2. "The central atom of the oxidant must be able to coordinate at least two hydroxyl groups in addition to groups already attached to it.

3. "The valence of the central atom must exceed by two units, rather than by one or three, the valence of the next lowest stable state.

4. "The oxidant must have an E_0 oxidation potential in the neighborhood of about -1.7 volts with respect to the next lowest stable valence state."

In general such oxidants are pictured[165] as operating by the formation of an ester with the glycol, the ester being decomposed with the oxidant liberated in its lower state of valence, and the remaining free radical rearranging to the dialdehyde:
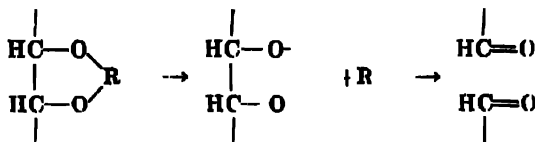
For periodate and lead tetraacetate, the intermediate complexes are too

[161] See comments by J. W Green, ref. 127, p. 180.
[164] I. J. Heidt, E. K. Gladding and C. B. Purves, *Paper Trade J.*, **181**, 81 (1945)
[165] R. Criegee, L. Kraft and B. Rank, *Ann.*, **507**, 159 (1933).

unstable to enable isolation, but with similar materials crystalline esters have been obtained (thallic esters of fatty acids and potassium osmate).

An alternative mechanism for this type of oxidation is based on a free radical mechanism.[166] Lead tetraacetate, for example, decomposes in hot solution according to the following equation:

$$Pb(OCOCH_3)_4 \rightarrow Pb(OCOCH_3)_2 + 2 \cdot OCOCH_3$$

The neutral acetate radicals may dehydrogenate 1,2-glycols as follows:
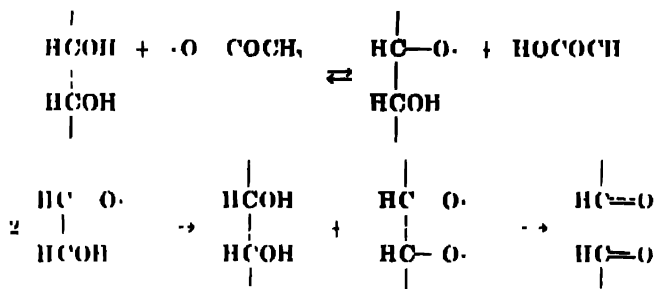
Periodic acid and lead tetraacetate are the most important agents of this type, but sodium perbismuthate ($NaBiO_3$) and hydrated trivalent silver ion (Ag^{+++}) also possess the necessary properties and oxidize glycols in a similar manner.

Periodic acid has its principal value for analytical and structural determinations. Lead tetraacetate is used for structural determinations (Chapter V) and preparatory purposes (Chapter III). For the latter purpose, sodium perbismuthate shows considerable promise because the original material and its reduction products are difficultly soluble in water and may be easily separated from reaction products of the glycol.

Cis 1,2-glycols are oxidized more rapidly than trans glycols by both lead tetraacetate and periodic acid, but the former reagent manifests such a marked difference in rate for cis and trans groups that it has been used for their estimation (pp. 213 and 365). Lead tetraacetate, because of its ease of hydrolysis, is usually employed in organic solvents, whereas aqueous solutions of periodic acid are used. Toward oxalic acid and α-hydroxy acids, the two reagents exhibit a marked difference. Oxalic acid is not attacked by periodic acid, and α-hydroxy acids are oxidized only slowly even at high temperatures. In contrast, lead tetraacetate attacks oxalic acid and α-hydroxy acids at room temperature.[167]

Periodic acid is an extremely valuable reagent. When hydroxyl groups, or an amino and a hydroxyl group, are located on neighboring carbon

[166] W. A. Waters, Trans. Faraday Soc., 42, 184 (1946).

[167] R. Criegee, Sitzber. Ges. Beförder. ges. Naturw. Marburg, 69, 25 (1934); Chem. Abst., 29, 6820 (1935).

atoms, cleavage of the intermediate carbon-carbon bond occurs upon treatment with periodic acid:

$$R'CHOH-CHOH-R + HIO_4 \rightarrow R'CHO + RCHO + HIO_3 + H_2O$$
$$R'CHNH_2-CHOH-R + HIO_4 \rightarrow R'CHO + RCHO + HIO_3 + NH_3$$
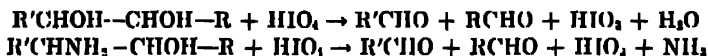
In many cases, the reactions are practically quantitative.

The use of periodic acid as a reagent for glycols was first applied by Malaprade. In the glycol series, Fleury and associates[168] showed that it is specific for 1,2-diols. The general application of the reagent has been reviewed by Jackson.[169]

The oxidation appears to take place through the intermediate formation of an unstable ester. Criegee[165] postulated the reaction as:
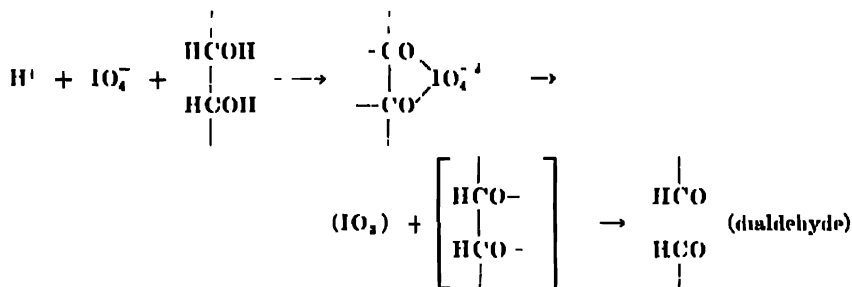
The ester formation is analogous to the formation of hydrates by periodate ions:

$$H^+ + IO_4 + 2H_2O \rightarrow 5H^+ + IO_6{}^5$$

The general conditions necessary for cleavage of carbon-carbon bonds has been discussed earlier in this chapter. Lead tetraacetate behaves similarly in many ways.

The oxidation with periodate is second order with respect to polyol and periodate.[170] It proceeds more rapidly in acid solution than in alkaline solution. Cis pairs of hydroxyls react more rapidly than trans groups. The effective oxidation potential in acid solution is about -0.8 volts.[161]

When more than two vicinal hydroxyl groups are available, the oxidation continues through this portion of the molecule with the formation of formic acid from secondary alcoholic groups and formaldehyde from primary alcoholic groups.

$$CH_2OH-(CHOH)_n-CH_2OH \xrightarrow{HIO_4} nHCOOH + 2HCHO$$

[168] P. Fleury and J. Lange, Compt. rend., 195, 1395 (1932).

[169] E. L. Jackson, in "Organic Reactions," Vol. 2, p. 341; John Wiley, New York (1944).

[170] C. C. Price and H. Kroll, J. Am. Chem. Soc., 60, 2726 (1938); C. C. Price and M. Knell, ibid., 64, 552 (1942).

Compounds containing carbonyl and hydroxyl groups are oxidized.

$$RCO—CH_2OH \rightarrow RCOOH + HCHO$$
$$RCO—CHO \rightarrow RCOOH + HCOOH$$

Aldonic and saccharic acids yield glyoxylic acid.[168, 171]

$$CH_2OH-(CHOH)_4-COOH + 4HIO_4 \rightarrow$$
$$CHO-COOH + HCHO + 3HCOOH + H_2O + 4HIO,$$

$$COOH-(CHOH)_4-COOH + 3HIO_4 \rightarrow$$
$$2CHO—COOH + 2HCOOH + H_2O + 3HIO_4$$
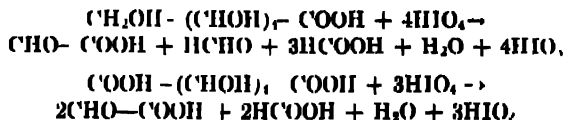
Glucose consumes five atoms of oxygen producing one mole of formaldehyde and five moles of formic acid. Inositol (a hexahydroxycyclohexane) is oxidized to formic acid and glycolic acid, 4 moles of oxidant being consumed rapidly and 6.7 moles at the end of several days.

In a carbon chain, the reaction stops when a carbon atom is reached which does not carry an unsubstituted hydroxyl, a carbonyl, or an amino group. Glycosides, for example, gives dialdehydes:
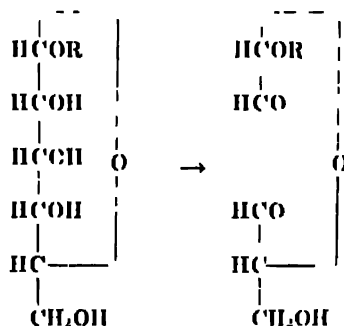
The amount of oxidant consumed as well as the nature of the reaction products provide proof for the structure of the glycoside. (For further discussion, see under structure of glycosides, Chapter V.) Periodic acid oxidation also provides information of great value for the determination of the structures of glycosans, ether derivatives, oligo- and polysaccharides (Chapters VIII, X and XII). It is also an important method of correlating the configuration of the anomeric carbon atoms, particularly in glycosides (p. 49, Chapter II).

In several instances, 1,2-glycols have been found to be resistant to the action of periodic acid. These include 1,6-anhydroglucofuranose[171a] and tetraacetylinositol.[171b] Hence, lack of oxidation by periodic acid cannot be taken as conclusive evidence of the absence of 1,2-glycol groups.

As a preparatory method, oxidation with periodic acid is of particular importance for the preparation of short-chain sugars. For example, 2,3-

[171] P. Fleury and J. Lange, J. pharm. chim., [8] 17, 313 (1933); P. Fleury, G. Poirot and J. Fievet, Compt. rend., 220, 664 (1945)

[171a] R. J. Dimler, H. A. Davis, and G. E. Hilbert, J. Am. Chem. Soc., 68, 1377 (1946).

[171b] G. Dangschat and H. O. L. Fischer, Naturwissenschaften, 30, 146 (1942).

benzylidene-D-arabitol (I) consumes one mole of periodic acid and yields 2,3-benzylidine-D-threose (II) which can be converted to a crystalline isopropylidene derivative.[172] The use of lead tetraacetate for this purpose is discussed elsewhere (p. 124).

$$
\begin{array}{ccc}
CH_2OH & & CH_2OH \\
| & & | \\
-OCH & & -OCH \\
| & & | \\
HCOCHC_6H_5 & \xrightarrow{HIO_4} & HCOCHC_6H_5 \\
| & & | \\
HCOH & & CHO \\
| & & \\
CH_2OH & & \\
(I) & & (II)
\end{array}
$$
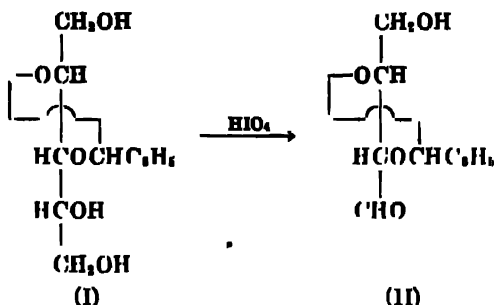
C. Nitric Acid and Nitrogen Oxides. Oxidations with nitric acid under the best conditions convert primary alcoholic and aldehydic groups to carboxylic groups. Frequently, however, cleavage of carbon-carbon bonds occurs. For galactose the conversion to insoluble mucic acid, COOH (CHOH)₄ COOH, takes place to an extent greater than 70%, and the reaction is used for the quantitative determination of this sugar.[173] Smaller yields of saccharic acid are obtained from glucose, and considerable quantities of oxalic acid and some tartaric acid are obtained.[174] Among the products of the oxidation of fructose are formic acid, oxalic acid, meso-tartaric acid, and glycolic acid, but the reaction seems to require more severe conditions than for glucose, and with dilute acid (32%) and low temperatures the ketoses are not attacked.

$$
\begin{array}{ccc}
CHO & & COOH \\
| & & | \\
(CHOH)_4 & \xrightarrow{HNO_3} & (CHOH)_4 \\
| & & | \\
CH_2OH & & COOH \\
(\text{D-Galactose}) & & (\text{Mucic acid})
\end{array}
$$
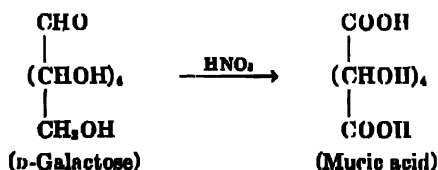
Oxidation of methylated sugars with nitric acid has been used extensively for the purpose of demonstrating the position of unsubstituted hydroxyl groups. (See under structure of glycosides and sugars such as maltose, sucrose, etc.)

Cleavage of carbon-carbon bonds appears to be facilitated by the pres-

---

[172] W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 1663 (1943)

[173] See C. A. Browne and F. W. Zerban, "Sugar Analysis," pp. 691,728, John Wiley, New York (1941); A. W. van der Haar, "Monosaccharide and Aldehydsäuren," Borntraeger, Berlin (1920).

[174] H. Kiliani, *Ann.*, **205**, 163, 172 (1880); *Ber.*, **54**, 463 (1921); W. E. Stokes and W. E. Barch, U. S. Patent 2,257,284, Sept. 30, 1941.

ence of catalysts such as vanadium salts, and tartaric and oxalic acids are formed in good yields at the expense of saccharic acids.[175] Since hot nitric acid acts as a hydrolyzing agent as well as an oxidizing agent, oligo- and poly-saccharides may be used.

Kiliani[176] has made an extensive study of the nitric acid oxidations of carbohydrates. He found that aldoses are oxidized to aldonic and saccharic acids or their lactones. Glucose, for example, gives gluconic acid and saccharic acid. Polyols can be oxidized to aldonic acids; glycerol gives glyceric acid. Aldonic acids are oxidized to 2-keto acids, saccharic acids, and uronic acids.[177] The formation of these products indicates that the oxidation of aldoses without cleavage of carbon-carbon bonds probably proceeds through the following series of reactions:

$$
\begin{array}{cccccc}
\text{CHO} & & \text{COOH} & & \text{COOH} & & \text{COOH} \\
| & & | & & | & & | \\
(\text{CHOH})_n & \rightarrow & (\text{CHOH})_n & \rightarrow & (\text{CHOH})_n & \rightarrow & (\text{CHOH})_n \\
| & & | & & | & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CHO} & & \text{COOH}
\end{array}
$$
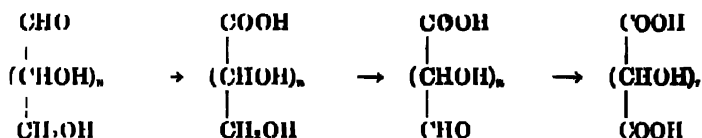
Alternatively, the reaction may proceed via the ring forms of the sugars and the lactones, but this refinement of the mechanism has not been clarified. Under the strongly acidic conditions of these oxidations, equilibria between the various ring and open-chain forms should be established quickly. In this connection, it should be noted that whereas galactose gives mucic acid (the open chain form), mannonic acid gives a dilactone.

The formation of 2-keto or 5-keto aldonic acids indicates that cleavage of carbon-carbon bonds may result from further oxidation of such intermediates. Vanadium salts appear to promote this reaction.

$$
\begin{array}{ccc}
\text{COOH} & & \text{COOH} \\
| & & | \\
\text{CO} & & \text{COOH} \\
| & & + \\
\text{CHOH} & \xrightarrow{\text{HNO}_3} & \text{COOH} \\
| & & | \\
\text{CHOH} & & \text{CHOH} \\
| & & | \\
\text{CHOH} & & \text{CHOH} \\
| & & | \\
\text{CH}_2\text{OH} & & \text{COOH}
\end{array}
$$

2-Keto aldonic acid   Oxalic acid and Tartaric acid
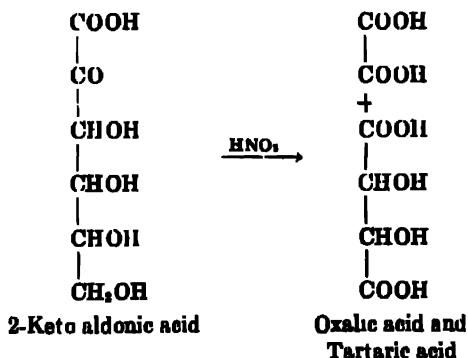
[175] J. K. Dale and W. F. Rice, Jr., *J. Am. Chem. Soc.*, **55**, 4984 (1933); S. Soltzberg, U. S. Patent 2,380,196, July 10, 1945; A. F. Odell, U. S. Patent 1,425,605, Aug. 15, 1922.
[176] H. Kiliani, *Ber.*, **54**, 456 (1921); **55**, 75, 2817 (1922); **56**, 2016 (1923); **58**, 2344 (1925).
[177] H. Kiliani, *Ber.*, **54**, 456 (1921), **55**, 75 (1922); W. Militzer and R. Angier, *Arch. Biochem.*, **10**, 291 (1946).

The specificity of the oxidation may be increased by the use of nitrogen dioxide (NO_2) rather than nitric acid.[178] With this reagent in gaseous form or in nonaqueous solution, a marked specificity for the oxidation of primary alcoholic groups (in the absence of aldehyde groups) has been shown. Glycosides are oxidized to uronic acids, cellulose to a polyglucuronic acid, and diethylacetal to glyoxylic acid.

$$\begin{array}{ccc} \text{R} & & \text{R} \\ | & \xrightarrow{\text{NO}_2} & | \\ \text{CH}_2\text{OH} & & \text{COOH} \end{array}$$
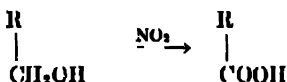
The nature of the oxidant in such systems has received some study.[179] Concentrated nitric acid exhibits an initial period of inhibition when used as an oxidizing agent and will not exert an oxidizing action in the presence of urea which removes nitrous acid. This period may be eliminated by the addition of fuming nitric acid, oxides of nitrogen, nitrous acid or other materials.[179] [180] Nitrogen dioxide appears to require the presence of water for its reaction. These facts indicate that nitric acid is not the true oxidant, but instead the effective agent is nitrous acid which in the presence of NO establishes an equilibrium[181] with nitric acid according to the equation:

$$\text{HNO}_3 + 2\text{NO} + \text{H}_2\text{O} \rightleftarrows 3\text{HNO}_2$$

The catalytic effect of oxides of nitrogen and of sodium nitrite appears to operate by the establishment of the above equilibrium. The action of nitrogen dioxide may be similar, for in the presence of water, a similar equilibrium condition is reached:

$$2\text{NO}_2 + \text{H}_2\text{O} \rightleftarrows \text{HNO}_3 + \text{HNO}_2$$

By the employment of conditions favorable for the establishment of these equilibria and unfavorable for carbon-carbon bond cleavage, the specificity of the reaction is increased greatly. For example, mucic acid is produced in 90% yield,[179] whereas with hot nitric acid the usual yield is about 75%.

Oxidation of primary alcoholic groups appears to take place through the intermediate formation of an ester of nitric (or nitrous) acid.[182] In the initial

[178] K. Maurer and G. Drefahl, *Ber.*, 75, 1489 (1942); K. Maurer and G. Reiff, *J. makromol. Chem.*, 1, 27 (1943); E. C. Yackel and W. O. Kenyon, *J. Am. Chem. Soc.*, 64, 121 (1942); C. C. Unruh and W. O. Kenyon, *ibid.*, 64, 127 (1942).

[179] B. L. Browning, C. R. Calkins, R. L. Leaf, Jr., W. H. McPherson and W. W Pigman, Abstracts, Am. Chem. Soc Meeting, New York, September (1947).

[180] H. Kiliani, *Ber*, 54, 456 (1921); G. D. Hiatt, U. S. Patent 2,256,391, Sept. 16, 1041; J. G. M. Bremner, R. H. Stanley, D. G. Jones and A W. C. Taylor, U. S. Patent 2,389,950, Nov. 27, 1945

[181] V. H. Veley, *Trans. Roy. Soc. (London)*, 52, 27 (1893); *J. Chem. Soc.* (Abstracts) 64, 413 (1893).

[182] P. A. McGee, W. F. Fowler, Jr., E. W. Taylor, C. C. Unruh and W. O. Kenyon, *J. Am. Chem. Soc.*, 69, 355 (1947).

stages of oxidation, cellulose contains combined nitrogen which increases and then slowly decreases. Nitric acid appears to act as a catalyst for the deesterification.

D. Oxygen in Alkaline or Neutral Solution. The study of the action of molecular oxygen on sugars is of considerable interest from the standpoint of the mechanism of the *in vivo* oxidations of sugars. The reaction also has considerable value for the degradations of sugars to acids having shorter chains (see Chapter III).

In alkaline solution, oxygen degrades the sugars to aldonic acids having one carbon atom less than the sugar. Air or oxygen may be used and relatively high yields of acids are obtained.[183] For example, potassium D-arabonate has been obtained from D-glucose in a yield of 60 to 75%. Ketoses act similarly, and in the case of L-sorbose, 2-keto-L-gulonic acid and L-xylonic acid are produced in good yield.[184] The formation of 2-ketogulonic acid indicates that the reaction proceeds through the osone, probably formed from the enediol:

$$
\begin{array}{ccccc}
\text{CHO} & \text{HCOH} & \text{HCO} & \text{OCOH} & \text{HCOOH} \\
| & || & | & | & | \\
\text{CHOH} \xrightarrow[\text{0-20°C.}]{\text{KOH}} & \text{COH} - \xrightarrow{\text{O}_2} & \text{CO} \xrightarrow{\text{O}_2} & \text{CO} \xrightarrow[\text{O}_2]{\text{H}_2\text{O}} & \text{OCOH} \\
| & | & | & | & | \\
\text{R} & \text{R} & \text{R} & \text{R} & \text{R}
\end{array}
$$
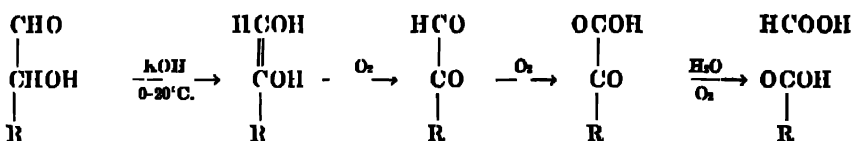
In neutral solution in the presence of platinum catalyst, the process is mainly one of dehydrogenation, and the oxidation of mannose is postulated[185] as follows:

Mannose → mannonic acid → mannuronic acid → *manno*-saccharic acid.

For fructose, the reaction seems to proceed differently:

Fructose → glucosone → 2-ketomannonic acid → D-arabinose.

The platinum oxide catalyst converts the hexitols to the corresponding aldoses and ketoses which are carried through the above series of reactions by the oxygen. Mannitol is oxidized by PtO_2 to D-mannose, isolated as methyl α-mannoside in a yield of 20%. Fructose is formed simultaneously.

Under conditions simulating biological processes (neutrality and a temperature of 37.5° C.), oxygen attacks glucose, glyceraldehyde, glycerol, and related products.[186] One mole of carbon dioxide is formed per mole of D-glucose. Sodium ferropyrophosphate is used as catalyst. With phosphate and

[183] J. U. Nef, *Ann.*, *403*, 204 (1914); O. Spengler and A. Pfannenstiel, *Z. Wirtschafts-gruppe Zucker ind.*, *85*, 546 (1935).
[184] O. Dalmer and K. Heyns, U. S. Patent 2,190,377, Feb. 13, 1939; H. S. Isbell, *J. Research Natl. Bur. Standards*, *29*, 227 (1942).
[185] J. W. Glattfeld and S. Gershon, *J. Am. Chem. Soc.*, *60*, 2013 (1938).
[186] H. A. Spoehr and H. W. Milner, *J. Am. Chem. Soc.*, *56*, 2068 (1934).

arsenate as catalysts, fructose is much more sensitive than glucose, and the rate of oxidation is dependent upon the concentration of salt present and not on the pH.[187]

In the absence of catalysts, alkaline solutions of aldonic acids and glykitols are relatively stable to oxygen. However, in the presence of salts of iron, nickel, cobalt, and copper, oxygen is consumed.[188] Carbon dioxide and formic acid are among the oxidation products.

From the standpoint of the conditions encountered during the manufacture of sucrose, the action of oxygen on sucrose solutions in the presence and absence of lime is important. Carbon dioxide is liberated from hot neutral solutions, and acids are formed. The increase in acidity results in inversion of the sucrose and decomposition of the resulting hexoses. The presence of lime or an increase in alkalinity speeds up the decomposition.[189]

E. Hydrogen Peroxide. The principal value of oxidations with hydrogen peroxide is for the degradation of aldonic acids to sugars with one less carbon atom; ferric sulfate is used as a catalyst (see p. 121). It is noteworthy that although ferric salts catalyze this reaction, ferrous salts are used for sugars and ferric salts are not effective.

With sugars, the products obtained depend upon the conditions employed and the presence and nature of the catalyst. In any case, the products are usually mixtures. At low temperatures and in the presence of ferrous sulfate, glucose and fructose are converted to glucosone and on further oxidation to glycolic acid, glyoxylic acid and trihydroxybutyric acid.[190] At low temperatures in the absence of catalysts, oxidation is very slow, but at high temperatures[191] the main product is carbon dioxide with some formic acid.

The nature of the products formed under various conditions and the mechanism of the reaction have been investigated by Küchlin.[192] At low temperatures and for dilute solutions in the presence of ferrous sulfate the following products were formed from glucose and identified as derivatives: glucosone, 2-ketogluconic acid, and 2,3-diketogluconic acid; in concentrated solutions, formaldehyde also was found. The formation of these products at low temperatures was ascribed to the following series of reactions:

[187] M. Clinton, Jr., and R. Hubbard, *J. Biol. Chem.*, *119*, 467 (1937)

[188] See: W. Traube and F. Kuhbier, *Ber.*, *69*, 2664 (1936).

[189] See: M. Garino, M. Parodi and V. Vignolo, *Gazz. chim ital.*, *65*, 132 (1935); *Chem. Abst.*, *29*, 5419 (1935).

[190] C. F. Cross, E. J. Bevan and C. Smith, *J. Chem. Soc.*, *73*, 463 (1898); R. S. Morrell and J. M. Crofts, *ibid.*, *75*, 786 (1899); *83*, 1284 (1903); H. A. Spoehr, *Am. Chem. J.*, *43*, 227 (1910).

[191] J. H. Payne and L. Foster, *J. Am. Chem. Soc.*, *67*, 1654 (1945); A. A. Kultyugin and L. H Sokolova, *Arch. sci. biol.* (*U.S.S.R.*), *41*, 145 (1936).

[192] A. Th. Küchlin, *Rec. trav. chim.*, *51*, 887 (1932) and earlier papers.

$$
\begin{array}{ccccccc}
\text{HCO} & & \text{HCO} & & \text{COOH} & & \text{COOH} \\
| & \xrightarrow{\;\frac{H_2O_2}{Fe^{++}}\;} & | & \xrightarrow{\;\text{dilute solution}\;} & | & \dashrightarrow & | \\
(\text{HCOH})_4 & & \text{CO} & & \text{CO} & & \text{CO} \\
| & & | & & | & & | \\
\text{CH}_2\text{OH} & & (\text{HCOH})_3 & & (\text{HCOH})_3 & & \text{CO} \\
& & | & & | & & | \\
& & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & (\text{HCOH})_2 \\
& & & & & & | \\
& & & & & & \text{CH}_2\text{OH} \\
(\text{I}) & \begin{array}{c}\text{conc.}\\\text{solution}\end{array}\Big\downarrow & (\text{II}) & & (\text{III}) & & \Big|(\text{IV})
\end{array}
$$

$$
\begin{array}{ccc}
\text{HCO} & & \text{HCHO} \\
| & & + \\
\text{CO} & \longrightarrow & \text{COOH} \longleftarrow \\
| & & | \\
\text{CO} & & \text{CO} \\
| & & | \\
(\text{HCOH})_2 & & (\text{HCOH})_2 \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
(\text{V}) & & (\text{VI})
\end{array}
$$
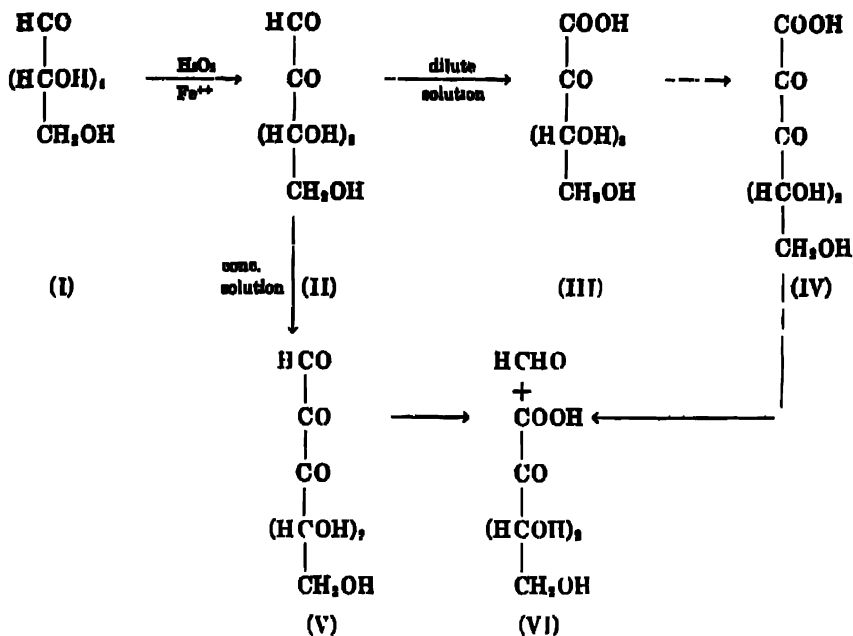
At higher temperatures, carbon dioxide, formic acid, oxalic acid, glycolic acid, tartronic acid, glyceric acid and other acids were shown to be formed. The formation of carbon dioxide is ascribed to decarboxylation of the 2,3-diketo acid (IV), and oxalic acid and trihydroxybutyric acid arise from cleavage of the C2-C3 bond. Compound V cleaves to glyoxylic acid and trihydroxybutyric acid. Compound VI is oxidized further to 2,3-diketosaccharonic acid which on cleavage gives oxalic acid and glyceric acid.

The catalytic effect of ferrous salts is ascribed by Kuchlin to the formation of a complex between ferrous ions and the carbonyl group and its neighboring hydroxyl group. This complex is oxidized, and the ferrous ion is converted to the ferric ion. Dissociation takes place, and the ferric ion is reduced to the ferrous state by further oxidation of the osones thus formed. Ferric ions will not catalyze the oxidation of sugars by hydrogen peroxide. Since ferric ions are used in the Ruff degradation of aldonic acids to sugars having one less carbon atom, ferrous ions if formed must be rapidly reoxidized by the hydrogen peroxide to ferric ions.

According to Haber and Weiss,[111] ferrous salts bring about the decomposition of hydrogen peroxide into free radicals:

$$Fe^{++} + HO\text{—}OH \longrightarrow Fe^{+++} + HO\cdot + (\text{:}O\text{—}H)^-$$

[111] See: W. A. Waters, *Ann. Repts. on Progress Chem. (Chem. Soc., London)*, **42**, 145 (1945).

Waters[189] suggests that the neutral hydroxyl radicals are the catalysts in ferrous ion catalyzed oxidations of α-hydroxy acids to 2-keto acids:

$$
\begin{array}{ccc}
\text{H} & & \text{OH} \\
| & & | \\
\text{R—C—COOH} + \cdot\text{OH} \rightarrow \text{R—C—COOH} \xrightarrow{\text{HO—OH}} & \text{R—C—COOH} \\
| & | & | \\
\text{OH} & \text{OH} & \text{OH} \\
& & \downarrow \\
& + \text{H}_2\text{O} & \text{R—C—COOH} + \cdot\text{OH} \\
& & \| \\
& & \text{O}
\end{array}
$$
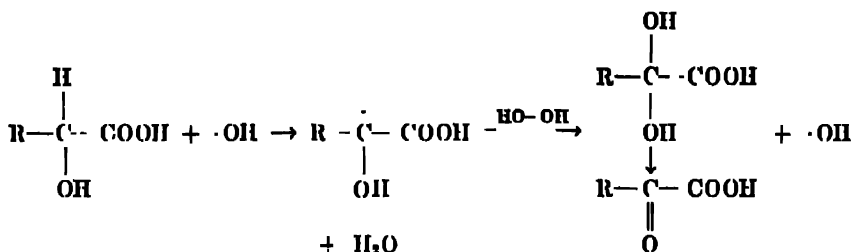
In the absence of catalysts, the oxidation may proceed by quite a different mechanism. Glucuronic acid has been prepared[194] in small yield by the oxidation of glucose with hydrogen peroxide without catalyst at 37° C. Kuchlin[192] provided evidence that the main products at moderately high temperatures are formic acid and tartronic acid. He explains this type of oxidation as proceeding through the steps of uronic acid formation, oxidative splitting-out of formic acid and repetition of the process.

$$
\begin{array}{ccc}
| & | & | \\
\text{HCOH} & \text{HCOH} & \text{HCOH} \\
| & | & | \\
\text{HCOH} \xrightarrow{\text{H}_2\text{O}_2} & \text{HCOH} \xrightarrow{\text{H}_2\text{O}_2} & \text{COOH} \\
| & | & | \\
\text{CH}_2\text{OH} & \text{COOH} & \text{HCOOH}
\end{array}
$$
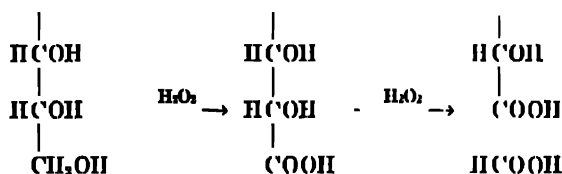
The formation of carbon dioxide is believed to be a secondary reaction. In the absence of catalysts, other carbohydrates probably are also oxidized initially at the primary alcoholic group.

The effect of variations in the conditions of the reaction have also been studied by Kuchlin.[195] For fructose with ferrous sulfate as a catalyst, the maximum velocity of reaction is between pH 3.2 and 5.1. The effect of an increase of temperature on the reaction is small in strongly acid solution but increases as the solution becomes more alkaline. The initial reaction velocity is proportional to the concentration of catalyst and is independent of the quantity of *ferric* salts. It is proportional to the hydrogen peroxide concentration.

Primary alcoholic groups are oxidized to aldehydes by peroxide and ferrous ions. Mannose (as the hydrazone) has been synthesized[196] from manni-

$$
\text{R—CH}_2\text{OH} \xrightarrow[\text{H}_2\text{O}_2]{\text{Fe}^{++}} \text{R—CHO}
$$

[194] A. Jolles, *Biochem. Z*, *34*, 242 (1911).
[195] A. Th. Kuchlin, *Biochem. Z.*, *261*, 411 (1933).
[196] H. J. H. Fenton and H. Jackson, *J. Chem. Soc.*, *75*, 1 (1899).

tol in yields of about 40%. In the absence of ferrous ions, even with ferric ions present, no reaction occurs. Presumably, the quantity of ferrous ions present is critical for it would be expected that with sufficient catalyst, the reaction would proceed farther as indicated above.

Everett and Sheppard[142] have studied the formation of reducing substances by the action of two molar equivalents of hydrogen peroxide on dilute solutions of gluconic δ-lactone at room temperature for a short time (30 minutes). Salts of K, Na, Li, Fe, Cu and Ni (carbonates, sulfates and acetates) catalyze the formation of reducing material. Of a number of anions tested, only bicarbonate, bismuthate, cyanate and tungstate ions exhibited catalytic action. Some of the observed effect may be due to changes in the hydrogen ion concentration.

Lactones and polyols showed increasing reducing power when treated with hydrogen peroxide in the presence of potassium bicarbonate. Sugars were not affected greatly, and uronic acids lost in reducing power. For the polyols, copper sulfate is more effective than potassium bicarbonate. For gluconic δ-lactone and copper sulfate or potassium bicarbonate as catalysts, acetate, fluoride and arsenate ions exert a synergistic effect manifested in an increased formation of reducing substances. On the other hand, many substances inhibit the reaction; iodides and p-aminobenzoates are example of such inhibitors.

F. Relatively Unspecific Oxidants. Most of the oxidants considered in previous sections will under some conditions produce general carbon-carbon cleavage and general oxidation of the various possible products. However, under carefully controlled conditions, it is possible to direct the reactions so that desirable products are obtained in appreciable yield. With other oxidants, the necessary conditions have not been established, except in special instances, and the products are carbon dioxide and a complex mixture of short chain compounds. This condition is particularly true for oxidations of reducing sugars. Among such oxidants are chromates, permanganates, silver oxide, copper sulfate, and cuprammonium, usually in alkaline solution. Interestingly enough, some of these oxidants are used for precise analytical work, under empirical but highly standardized conditions. (See also discussion of analytical methods in Chapter III.)

a. CHROMIC ACID AND CERIC SULFATE (ACID CONDITIONS)

Wet Combustions. Hot acid solutions of chromic acid and of ceric sulfate oxidize carbohydrate materials to carbon dioxide, formic acid and formaldehyde. The quantity of oxidant consumed constitutes a fairly precise measure of the amount of carbohydrate present.[197]

[197] G. Birstein and M. Blumental, *Bull. soc. chim.*, [5] *11*, 573 (1944); *Chem. Abst.*, *40*, 2437 (1946).

The equations for the oxidation of several sugars by ceric sulfate are:

$$2 \, C_6H_{12}O_6 \text{ (glucose)} + 13 \, O \rightarrow H_2O + CO_2 + 11 \, HCOOH$$
$$2 \, C_6H_{12}O_6 \text{ (fructose)} + 13 \, O \rightarrow 3 \, H_2O + 3 \, CO_2 + 7 \, HCOOH + 2 \, HCHO$$
$$C_{12}H_{22}O_{11} \text{ (sucrose)} + 13 \, O \rightarrow 2 \, CO_2 + 9 \, HCOOH + HCHO + 2 \, H_2O$$
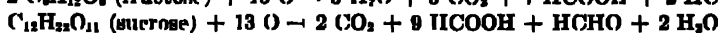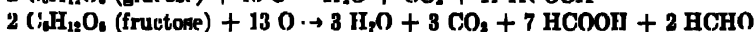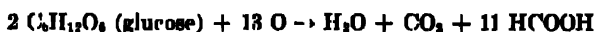
For sucrose, an accuracy of $\pm 0.3\%$ is claimed.

TABLE V

The Oxidation of Various Organic Compounds Using Alkaline Permanganate, Periodic, Sulfato Ceric, and Chromic Acids[197]

Compound	Formula	Alkaline KMnO₄	Periodic Acid	Sulfato-Ceric Acid	Chromic acid
Acetic Acid	CH₃COOH	X	X	X	X
Oxalic Acid	COOH·COOH			2	
Formic Acid	H·COOH	2	X	X	
Glycolic Acid	CH₂OH·COOH	6	X	3.95	
Malonic Acid	COOH·CH₂·COOH			6.66	
Tartaric Acid	COOH·CHOH·CHOH·COOH	10	2	7.20	
Succinic Acid	COOH·CH₂·CH₂·COOH			X	
Malic Acid	COOH·CHOH·CH₂·COOH	12	X	9 25	
Citric Acid	(CH₂COOH)₂·COH·COOH	18	X	15.85	
Pyruvic Acid	CH₃·CO·COOH			2	
Ethylene Glycol	CH₂OH·CH₂OH	(10)	2		
Glycerol	CH₂OH·CHOH·CH₂OH	(14)	4	6	14
Erythritol	CH₂OH·(CHOH)₂·CH₂OH	(18)	6		
Arabitol	CH₂OH·(CHOH)₃·CH₂OH			8	
Mannitol	CH₂OH·(CHOH)₄·CH₂OH	(26)	10		
Phenol	C₆H₅OH	(28)			
Salicylic Acid	C₆H₄·OH·COOH	(28)			
Gallic Acid	C₆H₂(OH)₃·COOH	24			
Formaldehyde	H·CHO	(4)	X		
Glucose	CH₂OH·(CHOH)₄·CHO	(24)	10		
Fructose	CH₂OH·(CHOH)₃·CO·CH₂OH	(24)	8		
Sucrose	C₁₂H₂₂O₁₁	(18)			
Ethyl Alcohol	C₂H₅OH		X		

Chromic acid acts similarly and has been used for the volumetric determination of cellulose materials.[199] Acid permanganate under the same conditions presumably would exhibit similar reactions.

$$C_6H_{10}O + 4(CrO_3)_x + 32H^+ \rightarrow 6CO_2 + 21H_2O + 8Cr^{3+}$$

A summary[198] of the effect of four oxidants on a number of organic materials is given in Table V. In the table X indicates no reaction. The figures

[198] See: H. F Launer, *J. Research Natl Bur. Standards, 20*, 87 (1938); *18*, 333 (1937)

[199] G. F. Smith, "Cerate Oxidimetry," G. F Smith Chemical Co., Columbus, Ohio (1942)

given are the number of equivalents of oxidant consumed. Whole numbers represent stoichiometrical reactions, and nonintegers, empirical reactions. Parentheses indicate results obtained by the improved permanganate method of Stamm.[200] Ceric sulfate oxidations were carried out by the procedure of Willard and Young[201] and perchlorate-ceric acid ($H_2Ce(ClO_4)_6$) by that of Smith and Duke.[202]

b. NEUTRAL AND ALKALINE PERMANGANATE

When relatively few functional groups are free, it is sometimes possible to use alkaline permanganate for the oxidation of specific groupings. 1,2-3,4-Diisopropylidenegalactose (I) can be oxidized[203] to the corresponding uronic acid (II):

(I)

(II)
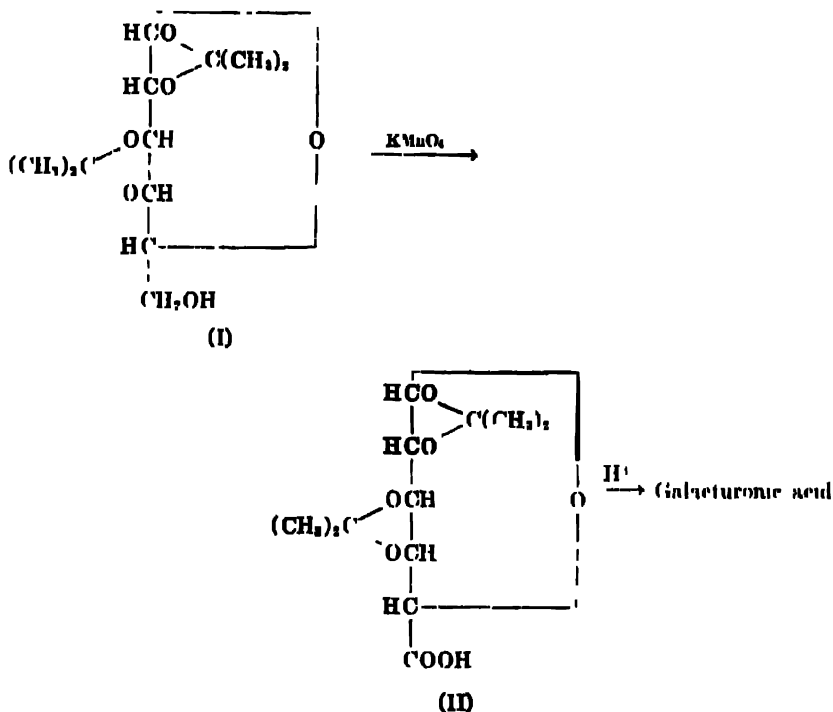
3-Methyl-L-xylonic acid is obtained from 1,2-isopropylidene-3-methyl-D-glucofuranose by permanganate oxidation and subsequent reduction of the xyluronic acid.[204]

[200] H. Stamm, Angew. Chem., 47, 791 (1934).

[201] H. H. Willard and P. Young, J. Am. Chem. Soc., 50, 1322 (1928).

[202] G. F. Smith and F. R. Duke, Ind. Eng. Chem., Anal. Ed., 13, 558 (1941).

[203] C. Niemann and K. P. Link, J. Biol. Chem., 104, 195, 743 (1934)

[204] W. Bosshard, Helv. Chim. Acta, 18, 956 (1935). See also examples under Keto Acids and Ascorbic Acids, earlier in this chapter and also p. 280

Glucose can be oxidized completely to carbon dioxide and water by hot alkaline solutions of potassium permanganate.[205] As the alkalinity increases above 0.03 N, oxalic acid is produced and in 1.8 N KOH, yields of 42% of oxalic acid are obtained.[206] Similar results are obtained from other hexoses, pentoses and glyceraldehyde.[207] The ratio of carbon dioxide to oxalic acid differs for various sugars, but at high temperatures the differences become small. The polyols are oxidized to the same products as the sugars, and the effect of alkalinity is the same as for the corresponding sugars.[208] Hence, it would appear that the sugars are intermediate products in the oxidation. The equivalents of oxidant required for several carbohydrates are given in Table V.

Alkaline permanganate at 0° acting on saccharic acid gives small yields of tartaric acid.[209]

In neutral or slightly acid solution, at room temperature, the ease of reactivity[210] of a number of sugars to permanganate is:

Maltose > fructose > arabinose > galactose > mannose > glucose > lactose; β-glucose > α-glucose.

For fructose, the maximal rate of oxidation takes place at pH 3.5 to 4.5

c. SILVER OXIDE

Aldohexoses, fructose, arabinose, erythritol, glyceraldehyde, saccharic acid and galactonic lactone are oxidized by silver oxide at 50° C (in water or N KOH) to carbon dioxide, oxalic acid, formic acid and glycolic acid.[211]

d. COPPER SALTS IN ALKALINE SOLUTION

The most important methods for the quantitative determination of reducing sugars are based on oxidation with hot alkaline solutions of copper salts (see Chapter III). The composition of the oxidation products has been investigated; in general, monobasic acids with one to six carbon atoms are formed accompanied by oxalic acid, carbon dioxide and lactic acid.

Copper sulfate in sodium carbonate solution (Solduini's reagent) oxidized glucose (at 100° C. for 8 hours) to a mixture of acids, more than 60% of which are non-volatile. In the non-volatile fraction, the following acids were identified: gluconic, mannonic, D-arabonic, erythronic, threonic, glyceric

[205] A. Smolka, *Monatsh.* **8**, 1 (1887)

[206] E. J. Witzemann, *J. Am. Chem Soc.*, **38**, 159 (1916).

[207] W. L. Evans and associates, *J. Am. Chem Soc.*, **47**, 3085 (1925); **47**, 3098 (1925).

[208] W. L. Evans and C. W. Holl, *J. Am. Chem. Soc.*, **47**, 3102 (1925).

[209] E. Fischer and A. W. Crossley, *Ber.*, **27**, 394 (1894).

[210] R. Kuhn and T. Wagner-Jauregg, *Ber.*, **58**, 1441 (1925).

[211] H. Kiliani, *Ann.*, **205**, 191 (1880); K. Dreyer, *ibid.*, **416**, 203 (1918). W. L. Evans and associates, *J. Org Chem*, **1**, 1 (1936)

and glycolic acids.[212] The same products are formed by the action of Fehling solution on glucose,[213] although the Fehling solution has a higher alkali concentration. From 199 g. of fructose, Nef reported the isolation of carbon dioxide (2.4 g.), formic acid (13.8 g.) and non-volatile acids (100 g) composed of: glycolic acid (22 g.), glyceric acid (18 g.), trihydroxybutyric acids (35 g.) and aldohexonic acids (30 g.). According to Nef,[214] the oxidation with copper acetate in neutral solution proceeds differently; much more oxygen is consumed, greater amounts of carbon dioxide are produced, and erythronic acid seems to be the main oxidation product.

In ammoniacal solutions of copper salts, the oxidation products are likely to contain nitrogen atoms.[215] Glucose, fructose, and mannose give oxalic acid, imidazoles, HCN and urea.

G. Microbial Oxidations.[216] Fermentative processes are of considerable value for the production of carbohydrate materials or closely related substances from carbohydrates. Large amounts of acetone, butyl alcohol, ethyl alcohol, acetic acid, lactic acid, citric acid, L-sorbose and gluconic acid are made industrially by fermentative methods. In fermentative processes, oxidizing as well as reducing conditions may be employed. Laboratory and industrial preparations of many other substances such as glycols have been carried out. The oxidation of polyols to ketoses is considered elsewhere as a preparatory method for ketoses (p. 131). Microorganisms exhibit a marked specificity in their choice of substrates and in the reaction products. This property is useful for the qualitative and quantitative determination of sugars (see p. 146), as well as for the identification of microorganisms. The formation of uronic acids and osones has been mentioned earlier. The present discussion will be limited to fermentative methods for the preparation of aldonic and keto aldonic acids.

Gluconic acid is produced by the action of many species of bacteria and molds on glucose.[217] Enzymes, glucose dehydrogenases, from molds, bacteria and liver, apparently bring about this reaction.[218] Boutroux found gluconic acid to be a metabolic product of acetic acid bacteria. Numerous bacteria and fungi oxidize glucose to gluconic acid, and the process is used for the

[212] F. W. Jensen and F. W. Upson, *J. Am. Chem. Soc.*, **47**, 3019 (1925).

[213] E. Anderson, *Am. Chem. J* , **42**, 40 (1909); J. U. Nef, *Ann.*, **357**, 214 (1907).   See also   J. Habermann and M. Honig, *Monatsh.*, **3**, 651 (1882); **5**, 208 (1884).

[214] J. U. Nef, *Ann* , **335**, 332 (1904); **357**, 259 (1907).

[215] J. Parrod and associates, *Compt. rend.*, **190**, 328 (1930); **192**, 1136 (1931); **200**, 1884 (1935), **212**, 610 (1941).

[216] For a general summary of the subject see: J. R. Porter, "Bacterial Chemistry and Physiology," p 896-1030, John Wiley, New York (1946).

[217] L. Boutroux, *Compt. rend.*, **91**, 236 (1880); A J Brown, *J. Chem. Soc.*, **49**, 172, 435 (1886).

[218] See: W. Franke and F. Lorenz, *Ann.*, **532**, 1 (1937).

commercial production of gluconic acid and its lactones and salts. Molds of species of *Aspergillus* and *Penicillium* are particularly suitable for large scale production. Using *Aspergillus niger* and fermentations under air pressure in rotating drums, yields of 90 to 99% of gluconic acid are obtained.[219] When calcium carbonate is present, calcium gluconate will crystallize directly.

Some strains of *A. niger* will oxidize D-mannose to mannonic acid and D-galactose to D-galactonic acid.[220] Many species of *Pseudomonas* and also *Acetobacter xylinum* will oxidize pentoses to the corresponding pentonic acids.[221] The yields are not always high but probably can be increased by improvements in the strains and in the cultural conditions.

Acetobacter suboxidans may oxidize glucose to 5-ketogluconic acid.[222] Gluconic acid is formed initially and only subsequently is converted to the 5-keto acid. 2-Ketogluconic acid is formed by some *Acetobacter* species, but *Pseudomonas* species give yields of over 80% after 25 hours when the solutions are strongly aerated in rotating drums.[223] (See also under Keto acids.)

[219] A. J. Moyer, P. A Wells, J J. Stubbs, H T. Herrick and O E. May, *Ind. Eng. Chem.*, **29**, 777 (1937), E A. Gastrock, N. Porges, P. A Wells and A J Moyer, *ibid.*, **30**, 782 (1938), N. Porges. T. F. Clark and S J Aronovsky, *ibid* **33**, 1065 (1941)

[220] H Knobloch and H Mayer, *Biochem Z*, **307**, 285 (1941)

[221] L B Lockwood and G E N Nelson, *J Bact*, **52** 581 (1946), G. Bertrand, *Compt rend.*, **127**, 124, 728 (1898).

[222] J. J. Stubbs, L B Lockwood, E T Roe, B Tabenkin and G E Ward, *Ind Eng. Chem*, **32**, 1626 (1940); A J Kluyver and A G J Boezaardt, *Rec trav chim*, **57**, 609 (1938); K R. Butlin and W H D. Winne, *J Soc Chem Ind*, **58**, 363 (1939).

[223] L B Lockwood, B Tabenkin and G E Ward, *J Bact*, **42**, 51 (1941).

CHAPTER VIII

ETHERS, ANHYDRIDES AND UNSATURATED DERIVATIVES

External and internal ether derivatives are known. The external ethers, particularly the methyl ethers, are important for the determination of the structures of sugars and polysaccharides. Inner ethers, also known as anhydrides, are produced under dehydrating conditions such as may occur during the esterification of glykitols. Both types of derivatives occur occasionally in natural products; methyl ethers of desoxysugars have been identified as the sugar portion of cardiac glycosides, and glykitol anhydrides (*e.g.*, styracitol) occur in plants.

Glycals and glycoseens are sugar derivatives containing a single double bond. For glycals this double bond is formed by the formal removal of two OH groups from carbon atoms 1 and 2; for the glycoseens H and OH are removed from neighboring carbon atoms with the formation of a double bond and not an anhydro ring. Unsaturated derivatives such as furfural, levulinic acid and ascorbic acid are considered elsewhere.

1. Ether Derivatives (External)

The application of the usual alkylating procedures of organic chemistry to the sugars and derivatives gives sugar ethers. Except for the alkoxyl group on carbon one of the aldoses (the glycosidic alkoxyl group in general), which is easily removed by acids, all the alkoxyl linkages are of the true ether type, and the groups are very resistant to removal. This property has made the sugar ethers, and in particular the methyl ethers, of great importance for the structural determination of the mono-, di- and polysaccharides. Simpler methods for determination of structure, particularly periodic acid oxidation, are now available for the simple glycosides and sugars, but the sugar ethers are still of great importance in serving as reference compounds in structural studies of the compound sugars. Although many methylated carbohydrates have been obtained in crystalline form, some of the more important ethers are known only as amorphous products. The use of the sirupy materials for identification purposes has, in a number of instances, led to erroneous assignments of structure.

Instances are known[1] of removal of methyl groups, but under most conditions they may be assumed to remain in their original position. Drastic treatment with hydrogen iodide or with HBr-acetic anhydride

[1] Sometimes a 2-methyl group is lost in osazone formation; P. Brigl and R. Schinle, *Ber.*, **62**, 1716 (1929); E. G. V. Percival and J. C Somerville, *J. Chem. Soc.*, 1615 (1937)

removes the alkyl groups.[2] The benzyl ethers of the sugars may be used to better advantage than the alkyl ethers when it is desired to remove ether groups since catalytic hydrogenation removes benzyl groups with the formation of toluene.[3] Trityl ethers (triphenylmethane ethers) are easily hydrolyzable and are widely used for the preparation of partially substituted derivatives of carbohydrates.

Polymethylated carbohydrates usually display considerable solubility in water as well as in organic solvents except aliphatic hydrocarbons. Partially methylated celluloses may exhibit some peculiar solubility relationships and be soluble in cold and insoluble in hot water (see under methylcelluloses).

Several natural sugar ethers are found among the products of hydrolysis of digitalis glycosides. These are 3-methyl-D-fucose (digitalose) and 2,6-didesoxy-3-methylallose (cymarose). Benzyl ethers of bacterial dextran have been patented for use in adhesives, lacquers and the like. Ethylcellulose is used industrially in various coating compositions and methylcellulose as a water-soluble protective colloid.

The allyl ethers of sugars and glycosides polymerize in the presence of oxygen. They are prepared best by treatment of glycosides with allyl bromide and alkali. Allyl tetraallyl-α-D-glucoside is a colorless sirup.[4]

A. Alkylation Methods. The most widely used procedure for alkylation depends on the action of dimethyl sulfate and 30% sodium hydroxide. Applied first by Denham and Woodhouse[5] to the methylation of cellulose, it was shown by Haworth[6] to be applicable to the simple sugars and glycosides. This method was utilized by Haworth, Hirst and associates in their extensive structural investigations. The procedure has the advantage of cheapness, of the solubility of the sugars in the reagents and of direct application not only to the glycosides but also to the sugars and to their acetyl derivatives. Acetyl groups are saponified under the conditions of the reaction and replaced by methoxyl groups. This modified procedure is of particular importance for the polysaccharides since the acetates are more
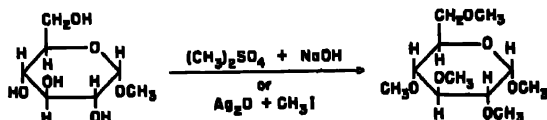
Methyl α-glucoside   Methyl tetramethyl-α-glucoside

[2] J. C. Irvine and A. Hynd, *J. Chem. Soc.*, **101**, 1145 (1912); K. Hess and F. Neumann, *Ber*, **68**, 1371 (1935).

[3] K. Freudenberg, H. Toepffer and C. C. Andersen, *Ber*, **61**, 1750 (1928).

[4] E. A. Talley, M. D. Vale and E. Yanovsky, *J. Am. Chem. Soc.*, **67**, 2037 (1945).

[5] W. S. Denham and H. Woodhouse, *J. Chem. Soc*, **103**, 1735 (1913).

[6] W. N. Haworth, *J. Chem. Soc.*, **107**, 13 (1915).

soluble in organic solvents than are the unsubstituted substances. Some improvements in the original procedure have been described.[7]

The well known reagent of Purdie (alkyl iodide and silver oxide) also may be applied to the alkylation of sugar derivatives.[8] Sugars with a free reducing group must be converted first to glycosides because of the oxidizing action of the silver oxide. Other limitations are the cost of the reagents, the insolubility of many sugar derivatives in the methyl iodide, and the number of treatments with the reagent, often six or more, necessary for complete methylation. By the addition of methyl alcohol, dissolution may be aided. The number of treatments required may be reduced by a preliminary application of the Haworth procedure.

In the above methods, particularly for sluggishly reacting compounds like pentamethylmannitol or partially methylated polysaccharides, much of the reagent is expended in forming methanol and methyl ether as a result of reaction of the alkylating agent with solvent or with by-products of the reaction (water). Often the methylation becomes very inefficient or fails to reach completion.

Modifications of the Williamson ether synthesis give better results than the above methods, but the synthesis is difficult to apply because of the low solubility of many carbohydrates in solvents inert to sodium.

$$2ROH + 2Na \rightarrow 2RONa + H_2$$
$$RONa + CH_3I \rightarrow ROCH_3 + NaI$$
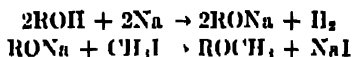
Freudenberg and Hixon[9] applied the Williamson synthesis to diisopropylidenefructose and prepared the sodium derivative by reaction with sodium in benzene solution. The sodium derivative reacted with methyl iodide to give 3-methyl-diisopropylidenefructose.

$$HCOH \xrightarrow{Na} HCONa \xrightarrow{CH_3I} HCOCH_3$$

A solvent of much greater versatility for the preparation of sodium derivatives of carbohydrates is liquid ammonia. Schmid and associates[10] showed that the liquid ammonia technique of Kraus and White[11] could be used to prepare sodium derivatives of carbohydrates. Muskat prepared the sodium derivatives of sugars; after removal of the liquid ammonia, the

[7] E. S. West and R. F. Holden, *J Am Chem Soc*, *56*, 930 (1934); J. Y. Macdonald, *ibid*, *57*, 771 (1935)

[8] T. Purdie and J. C. Irvine, *J. Chem. Soc*, *83*, 1021 (1903).

[9] K. Freudenberg and R. Hixon, *Ber.*, *56*, 2125 (1923); E. Pacsu and S. M. Trister, *J. Am. Chem. Soc.*, *61*, 2442 (1939)

[10] L. Schmid and B. Becker, *Ber*, *58*, 1966 (1925).

[11] C. A. Kraus and G. F. White, *J. Am. Chem. Soc.*, *45*, 769 (1923).

products were resuspended in an inert solvent and alkylated with methyl iodide. Potassium and lithium derivatives also were made by Muskat.[12]

The liquid ammonia method enables the completion of the methylation process often in three or four operations. With polyols, the mono- and disodium derivatives are so insoluble that it is preferable to commence with the Haworth procedure and then change to the liquid ammonia method.

Thallium salts of glycosides, made by treatment of glycosides with aqueous thallous hydroxide, react with methyl iodide to give methyl ethers.[13]

Diazomethane partially methylates starch, lichenin and inulin (30 to 50% of theory) but has no effect on cellulose.[14]

The hydroxyls other than the hemiacetal hydroxyl do not exhibit considerable preferential reaction with the Haworth reagent (methyl sulfate and alkali) or the Purdie reagent (silver oxide and methyl iodide) and partial methylation leads usually to mixtures. A better method of preparing partially methylated sugars consists in blocking all of the groups which are to be free in the final product, then methylating the compound and finally removing the blocking groups. Blocking groups must be able to withstand the methylating conditions without hydrolysis; for the Haworth procedure, the isopropylidene and benzylidene derivatives, stable to alkaline conditions and removed by acids, are often of value. The 3-methyl glucose (III) is synthesized by the methylation of 1,2 5,6-isopropylidene-glucofuranose (diacetone glucose) (I); as the only free hydroxyl is at carbon 3, the 3-methyl derivative (II) is formed Acid hydrolysis then removes the isopropylidene groups
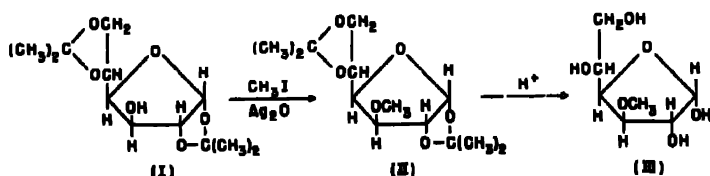
B. Trityl Derivatives.
Triphenylmethyl chloride, $(C_6H_5)_3C-Cl$, was shown by Helferich and associates[15] to react with sugars, glycosides and derivatives to form the triphenylmethyl ethers, commonly called trityl derivatives. The reagent exhibits a marked difference in the rate of reactivity for the primary and secondary alcoholic hydroxyls of the sugar molecule, and con-

[12] I E Muskat, *J Am. Chem Soc*, 56, 2449 (1934); S Soltzberg, U S Patent 2,234,200, March 11, 1938

[13] C. M Fear and R Menzies, *J Chem Soc*, 937 (1926)

[14] L Schmid, *Ber*, 58, 1963 (1925).

[15] B Helferich, P. E. Speidel and W Toeldte, *Ber*, 56, 766 (1923); B Helferich, *Z angew Chem*, 41, 871 (1928)

ditions often may be selected for bringing about reactions with the primary groups alone. For the hexose sugars, the 6-trityl derivatives are produced by reaction with trityl chloride or bromide in pyridine solution.[16]
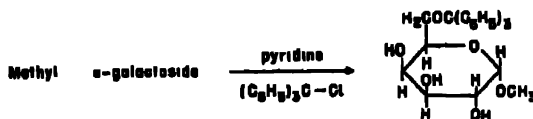
Under more prolonged treatment particularly when no primary alcoholic groups are present in the molecule, as for the methyl pentopyranosides and 6-desoxyhexosides, reaction with secondary hydroxyls occurs with the formation of mono- and di-trityl derivatives.[17] As shown by studies of the rate of oxidation with lead tetraacetate, the methyl monotrityl-β-L-fucoside has the trityl group attached to carbon 2. The two known methyl mono-trityl-β-D-arabinosides have the trityl groups at carbons 2 and 3 [18]

TABLE 1

Rate of Reaction of Sugar Derivatives with Trityl Chloride

Substance	Excess of Trityl Chloride	*k*
1 2 3 4-Diisopropylideneagalactopyranose	4 fold	0 014
	8 fold	0 036
2,3 4,6 Diisopropylidenesorbofuranose	4 fold	0 0052
	8 fold	0,0055
1,2-5,6-Diisopropylideneglucofuranose	4 fold	0 00012
	8 fold	0 00016

The rates of reaction of triphenylmethyl chloride with several character-istic compounds are given[19] in Table I, which also illustrates the effect of the trityl chloride concentration. For the 8-fold excess, the *primary* alco-holic group of the galactose derivative reacts 220 times as rapidly as the *secondary* alcoholic group of the glucose derivative. However, the difference between the *primary* alcoholic group of the sorbose derivative and the *secondary* hydroxyl of the glucose derivative is only 34 times.

This kinetic comparison is made between primary hydroxyl groups and ring secondary groups. Since a considerable portion of the difference may arise from steric factors, the difference between acyclic primary and

[16] D. D. Reynolds and W. L. Evans, *J. Am. Chem. Soc.*, **60**, 2559 (1935).

[17] R. C. Hockett and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 945 (1934); E L. Jack-son, R. C. Hockett and C. S. Hudson, *ibid.*, **56**, 947 (1934).

[18] R. C. Hockett and D F. Mowery, Jr., *J. Am. Chem. Soc.*, **65**, 403 (1943)

[19] R. C. Hockett, H. G. Fletcher, Jr, and J Ames, *J. Am. Chem. Soc.*, **63**, 2516 (1941).

secondary hydroxyl groups may not be as great, and it is possible that certain secondary hydroxyls, *e.g.*, that in isopropyl alcohol, may actually react faster than some primary hydroxyls.

The reagent reacts with both primary hydroxyls of fructose, but by use of the proper proportions, mono- or di-tritylfructose is formed.[20] All of the primary hydroxyls of di- and tri-saccharides react easily, and the reaction is sometimes used to determine the number of such groups in the molecule[21] (see also under Nucleosides).
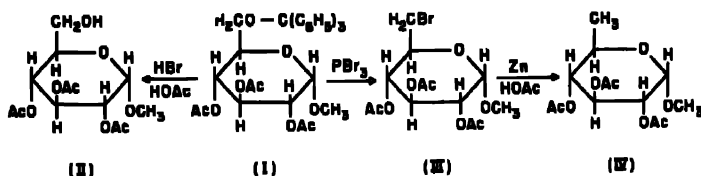
The trityl derivatives have their greatest value for the preparation of acetylated sugars in which the primary hydroxyl groups are unsubstituted (II) and for the corresponding halogen derivatives (III). The acetylaldohexoses with unsubstituted primary hydroxyls are important intermediates in the preparation of disaccharides of the gentiobiose type (see Chapter X) and of 6-methyl sugars.

2. Carbohydrate Inner Ethers (Anhydrides)[21a]

The carbohydrate inner ethers, also known as anhydrides, contain stable five- and six-membered rings formed by internal etherification between two nonglycosidic hydroxyl groups.

Compounds with five-membered rings are the most important. Alcohols, sugars, mono- and di-basic acids with this ring structure are known. Since the stereochemistry of the stable ether rings is common to all classes, it is convenient to treat them together. Particularly in the six-carbon series, the 3,6-anhydro ring governs the structure so that the anhydro ring takes on the character of the principal ring structure, to which the ordinary pyranose or furanose ring is only subsidiary.[22] In this important respect the ordinary sugar inner ethers differ from the glycosans as well as those of the ethylene oxide type. In the two latter types the characteristic ring is labile and, in the case of the glycosans and 1,2-epoxides, involves the principal carbonyl function. Compounds of all three types in the sugar series have been classed as anhydro sugars (see Glycosans).

[20] B. Helferich, *J. prakt. Chem*, [2] *147*, 60 (1936–37)

[21] K. Josephson, *Ann.*, *472*, 230 (1929).

[21a] This section was prepared by the late R. Max Goepp, Jr.

[22] W. N Haworth, L N. Owen and F. Smith, *J. Chem. Soc.*, *88* (1941).

A. Five-membered Rings. 1,4 and 3,6 Types. Erythritol gives a sirupy 1,4-anhydride on heating with dilute sulfuric acid or with phosphoric acid and subsequent saponification of the monophosphate ester.[21]

CH₂OH structures:
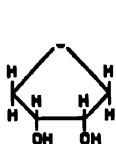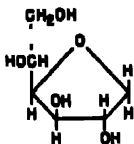
1,4-Erythritan   1,4-Sorbitan   1,4-Mannitan
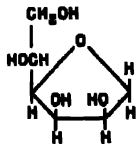
As shown by the early work of Bouchardat, Berthelot and Vignon, under etherifying or anhydridizing conditions as by heating with mineral acids, alkalies, or organic acids, the hexitols give rise to a complex mixture of inner ethers, including both mono- and di-anhydrides.[24] The monoanhydrides are known as glykitans. If only one mole of water is removed per mole of hexitol, crystalline 1,4-sorbitan can be obtained from sorbitol, and 1,4-mannitan from mannitol. With mannitol some external etherification occurs. Sirupy mono and dianhydro products of xylitol have been described.

The 3,6-sorbitan[25] and 3,6-mannitan[26] (identical with 1,4-mannitan) inherit the structural proof of their related anhydro sugars described later. The configuration of 1,4-sorbitan (also called arlitan) was derived from that of the well established 2,3,5,6-tetramethyl-D-glucofuranose, by reducing the furanose to the corresponding tetramethylsorbitol, and anhydridizing the latter to 2,3,5,6-tetramethyl-1,4-anhydrosorbitol. This was identical with tetramethylarlitan.[27]

1,4-Mannitan is also obtained, as a dibenzoyl derivative, by heating 1,6-dibenzoylmannitol in acetylene tetrachloride; migration of one of the benzoyl groups to the carbon 2 (or 5) takes place and other anhydro products are formed.[28]

[22] A. Henninger, *Ann. chim. phys.*, [6] 7, 224 (1886); P. Carré, *Ann. chim. phys.*, [8] 5, 345–432 (1905).

[24] See. "Beilsteins Handbuch der organischen Chemie", Vol. 1, p. 538–540 (1918) Also: J. Muller and U. Hoffmann, U. S. Patent 1,757,468, May 6, 1930; S. Soltzberg, U. S. Patent 2,390,395, Dec. 4, 1945; F. Grandel, U. S. Patent 2,375,915, May 15, 1945

[25] E. Fischer and K. Zach, *Ber.*, 45, 2068 (1912).

[26] F. Valentin, *Collection Czechoslov. Chem. Communs.*, 8, 35 (1936).

[27] S. Soltzberg, W. Freudenberg and R. M. Goepp, *J. Am. Chem. Soc.*, 68, 919 (1946).

[28] a. P. Brigl and H. Gruner, *Ber.*, 66, 1945 (1933); 67, 1582 (1934); b. R. C. Hockett, H. G. Fletcher, E. L. Sheffield, R. M. Goepp and S. Soltzberg, *J. Am. Chem. Soc.*, 68, 930 (1946).

Directed syntheses,[29] applicable to the sugars, involve removal by the action of alkali of 6-bromo, 6-tosyl, 6-sulfate, or 6-nitro groups from a methyl aldohexoside derivative containing a free or acetylated hydroxyl at carbon 3.
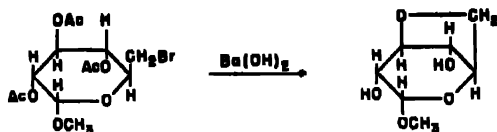
Methyl 6-bromo triacetyl α-D-glucoside   Methyl 3 6 anhydro α-D-glucoside

Although the 6-ring in the dicyclic anhydro glucoside is strained, the compound is distillable under diminished pressure. Acid hydrolysis yields the free 3,6-anhydroglucose, which is reducible to 3,6-sorbitan and oxidizable to 3,6-anhydrogluconic acid.[29a][c] The structure of 3,6-anhydroglucose was proved by its synthesis from 6-tosyl-1,2-isopropylidene-glucose, known to have a furanose structure and hence having only one available hydroxyl[29b] other than that at carbon 5. (The 5,6-anhydride is a different compound, see p. 361).
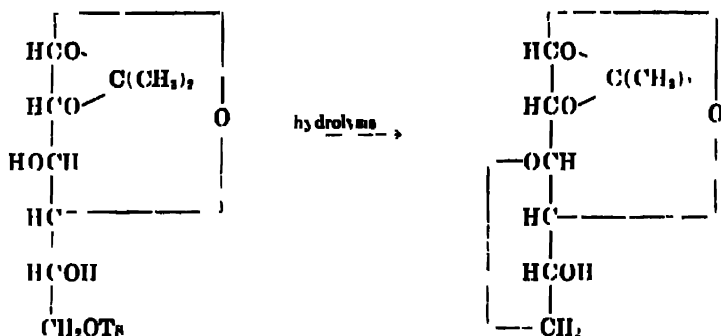
Applied to methyl α-D-mannopyranoside[25][29a] or to 1,5-mannitan,[30] the Fischer-Zach synthesis yielded the corresponding 3,6-anhydro derivative, although in this case the sterically possible 2,6-ring closure would have given a dicyclic structure with strainless six-membered rings.

Once formed, the stable, comparatively rigid five-membered ring in the 3,6-anhydro compounds strongly influences their class behavior. Where the hydroxyl on carbon four is available, as with compounds of the gluco

[29] a. E. Fischer and K. Zach, *Ber.*, **45**, 456 (1912); b. H. Ohle, L. von Vargha, and H Erlbach, *Ber.*, **61**, 1211 (1928); c. W. N. Haworth, J. Jackson and F. Smith, *J. Chem. Soc.*, 620 (1940); d. E. G. V. Percival, *J. Chem Soc.*, 119 (1945); e. E. K. Gladding and C. B. Purves, *J Am. Chem Soc.*, **63**, 76 (1944).

[30] R C Hockett and E L. Sheffield, *J. Am. Chem. Soc.*, **63**, 937 (1946)

and *manno* configuration, a second five-membered ring can be closed. Thus, 3,6-sorbitan and -mannitan give the 1,4-3,6-hexides (isosorbide[21] and isomannide);[28] 3,6-anhydroglucose and mannose give the furanose or methyl furanoside, and 3,6-anhydroglucuronic acid yields the γ-lactone[27]
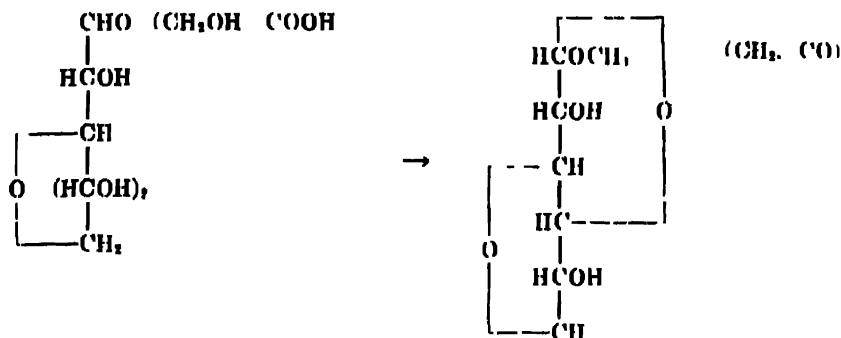
under the appropriate conditions. Free 3,6-anhydrogalactose, or the corresponding acid, however, cannot close the relatively strainless furanoside or gamma ring, since the hydroxyl at carbon 4 is now on the opposite side of the principal ring. Accordingly, the open chain forms are now the more stable, so that glycosidation yields the dimethyl acetal,[32] and the anhydrogalactonic acid shows little tendency to lactonize. In the case of the corresponding 2,4-dimethyl ether derivatives, however, the ring closure to the δ-lactone or to the methyl pyranoside can be forced. With the pyranoside, the driving force of conversion from the dimethyl acetal seems to be the much stronger crystallizing power of the methyl pyranoside.[32] The methyl 2,4-dimethyl-3,6-anhydro-α-D-glucopyranoside is isomerized directly to the methyl 2,4-dimethyl-3,6-anhydro-β-D-glucopyranoside without loss of methyl group. Furthermore, the methyl 3,6-anhydro-α and β-glucopyranosides can be changed to the corresponding furanoside forms, without formation of free sugar, and without change of configuration at carbon one. These transformations are carried out preferably in absence of water since aqueous sulfuric acid tends to form the free anhydroglucose.[33] The 3,6-rings are formed in the hydrolysis of the 3- and 6-sulfate esters of D-glucose derivatives even when it is possible to form ethylene oxide rings.[33]

The inner ether ring in methyl 3,6-anhydro-β-D-galactopyranoside may be split by vigorous acetolysis, using acetic anhydride and sulfuric acid, to give heptaacetyl-D,L-galactose.[34]

[21] L. F. Wiggins, *J. Chem. Soc.*, 4 (1945).
[28] W. N. Haworth, J. Jackson and F. Smith, *J. Chem. Soc*, 620 (1940)
[32] E. G. V. Percival, *J. Chem. Soc.*, 119 (1945).
[34] T. L. Cottrell and E. G. V. Percival, *J. Chem. Soc.*, 749 (1942).

B. Isohexides (1,4–3,6-Hexides). The 1,4–3,6-dianhydrides or iso-hexides of mannitol, sorbitol and iditol are obtainable by the direct acid-catalyzed anhydrization of the parent hexitols or of the intermediate 1,4 or 3,6-hexitans. This synthesis constitutes proof of structure for iso-sorbide.

```
   CH₂─┐            CH₂─┐             CH₂OH
    │   │            │   │             │
   HCOH │           HCOH │            HCOH
    │   O  ──→        │   O    ←        │
   HOCH │          ──CH │            ──CH
    │   │            │   │          │   │
   HC───┘          O HC─┘          O   HCOH
    │                │              │   │
   HCOH            HCOH               HCOH
    │                │                │
   CH₂OH          └──CH₂           └──CH₂
```

1,4 Sorbitan   1,4-3,6 Sorbide   3,6 Sorbitan
                    (isosorbide)
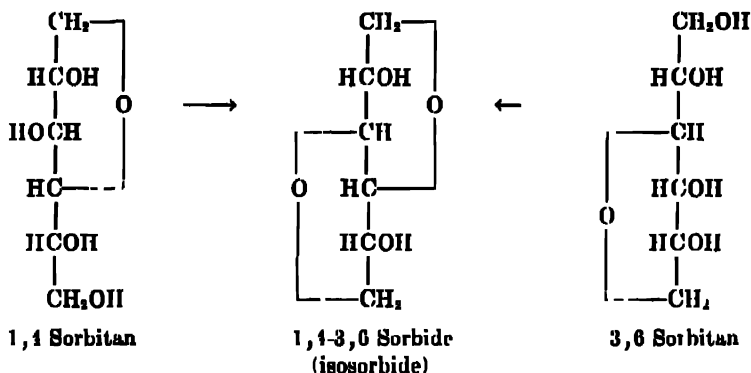
Independent proof of the 1,4–3,6 structure for isomannide has been given by Wiggins.[31] The corresponding 1,4 3,6-L-idide is likewise obtainable by the catalytic isomerization of both D-isomannide and D-isosorbide over Raney nickel, under hydrogen pressure at 200°C.[34a] As L-iditol is the only other hexitol epimeric with D-mannitol and D-sorbitol at carbons 2 and 5, this transformation affords proof of the idide configuration.

```
   CH₂─┐            CH₂─┐             CH₂─┐
    │   │            │   │             │   │
   HCOH │           HOCH │            HCOH │
    │   O            │   O             │   O
  ──CH             ──CH              ──CH
    │   │            │   │             │   │
   HC──┘           HC──┘             HC──┘
 O  │            O   │            O    │
    HCOH            HCOH              HOCH
    │                │                │
  └──CH₂          └──CH₂            └──CH₂
```

1,4-3,6-Dianhydro-D-   1,4-3,6-Dianhydro-D-   1,4-3,6-Dianhydro-
sorbitol (isosorbide)   mannitol (isomannide)   L-iditol
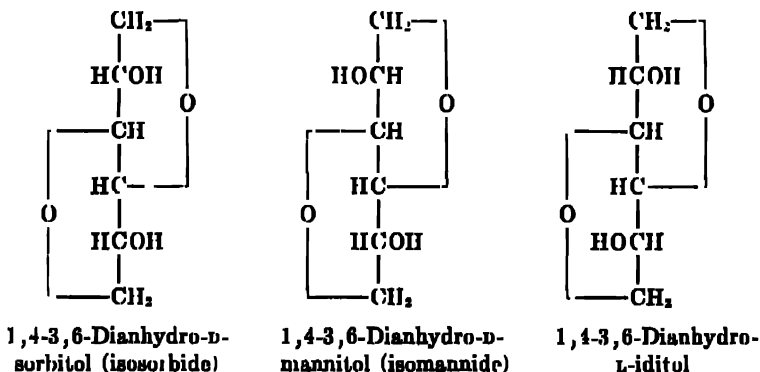
C. 2,5-Anhydro Compounds. Splitting out water from two secondary hydroxyl groups requires that one or the other contribute a hydroxyl group, hence that the carbon-oxygen bond be broken. In such a case, Walden inversion frequently occurs at the carbon atom concerned (see under Tosyl and Sulfate esters). With D-mannitol, symmetry is such that

[34a] H. G. Fletcher, Jr., and R. Max Goepp, Jr., *J. Am. Chem. Soc.*, **68**, 939 (1946).

inversion or epimerization at either carbon 2 or carbon 5 leads to the *gluco* configuration, or sorbitol. Accordingly, acid catalyzed 2,5-anhydrization

```
    CH₂OBz                      CH₂OBz                     CH₂OBz
      |                           |                          |
    HOCH                        HC———                      HC———
      |                           |     |                    |    |
    HOCH        -H₂O            HOCH    |      KMnO₄         CO    |
      |        ——————→           |     O    ———————→        |     |
    HCOH                        HCOH    |                 O   O    |
      |                           |     |                   \      |
(I) HCOH        (II)            HC———   |        (III)       CO     |
      |                           |                          |     |
    CH₂OBz                      CH₂OBz                      HC———
                                                             |
                                                           CH₂OBz
 1,6-Dibenzoyl-D-        2,5-Anhydrosorbitol
    mannitol              1,6-dibenzoate                      +

                                                           CH₂OBz
                                                             |
                                                           HC———
                                                             |     |
                                                        (HO)₂CH    |
                                                             |     O
                                                        (HO)₂CH    |
                                                             |     |
                                                           HC———
                                                             |
                                                     (IV)   CH₂OBz
```
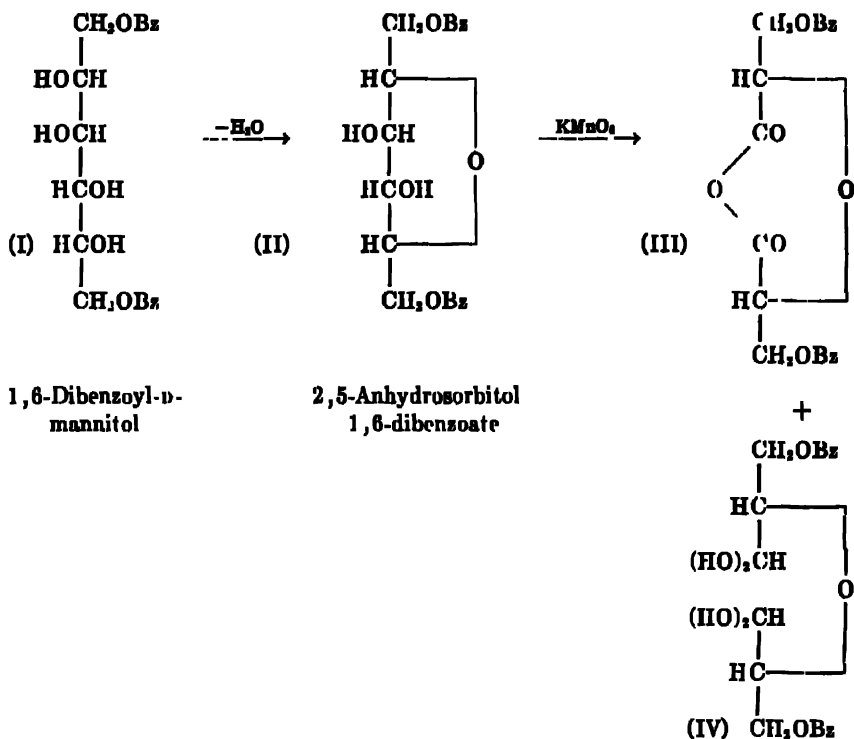
of 1,6-dibenzoylmannitol (I) would give rise solely to the 1,6-dibenzoyl-2,5-sorbitan (II), if the blocking groups on the primary hydroxyls did not migrate. A crystalline 2,5-hexitan 1,6-dibenzoate has in fact been obtained. The sorbitan configuration is made very probable by the permanganate oxidation of the hexitan diester (II) to an optically inactive ether glyceric acid anhydride diester (III), and a similarly inactive glyceraldehyde ether dibenzoate dihydrate, (IV). Destruction of asymmetry at carbons 3 and 4 allows (III) and (IV) to become inactive by internal compensation, and this is possible only if the C-O-linkages in (III) and (IV) are on the same side, as shown.[28a, 35]

The 2,5-anhydroiditol is known in the form of its 1-tosyl, 1,6-ditosyl, and 1,6-diiodo derivatives. It was obtained by the acid hydrolysis of 1-tosyl-2,4-benzylidene-5,6-anhydrosorbitol. Presumably the hydroxyl on carbon 2, set free by the hydrolysis, opens the ethylene oxide ring at 5,6. Since the ditosyl derivative of the anhydride is split by lead tetraacetate to

35 R. C. Hockett, M. Zief and R. M. Goepp, *J. Am. Chem. Soc.*, **68**, 935 (1946).

give an optically active product,[36] Walden inversion must have occurred
at carbon 5. It is noteworthy that the related 1,3-2,4-diethylidene-5,6-
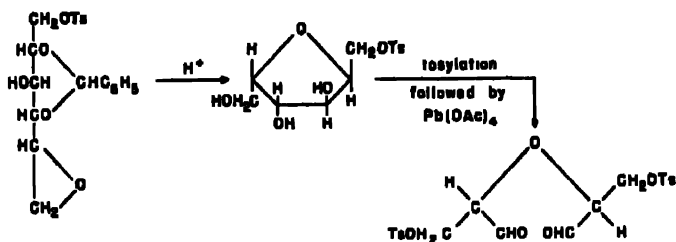
anhydrosorbitol is split by dilute acetic acid without anhydro formation.
In this case the ethylene oxide ring may be opened before the hydroxyl at
carbon 2 is set free

When ring closure is between a primary and a secondary hydroxyl, as
in the 3,6-anhydrides, rupture of the C-OH linkage could occur at either
the primary or the secondary hydroxyl, and inversion could follow only
rupture at the secondary hydroxyl. When a bromine atom or tosyloxy
group is split off from the terminal carbon the oxygen must remain attached
to the secondary carbon 3, as in the 3,6 series. Hence, no inversion can occur

The six-carbon sugars and mono and dibasic acids of the 2,5-anhydro
series are obtained by treating the open-chain 2-hexosamines and their
related aldohexonic acids with nitrous acid. Walden inversion at carbon 2
takes place in certain cases, but not in others, so that the final configura-
tions must be established by independent methods. Levene has made the
major contribution in this field, following the pioneering work of E.
Fischer.[37] The relationships between the various products is shown in
Table II.

Bromine oxidation of a hexosamine to the corresponding aldonic acid
amine (aldosaminic acid) allows deamination to proceed without inversion
at carbon two, giving the corresponding 2,5-anhydroaldonic acid, con-
vertible by nitric acid oxidation to the related 2,5-anhydrosaccharic acid.
If, however, the original amino sugar is deaminated, inversion occurs at
carbon two, and 2,5-anhydro sugars, further oxidizable by bromine to the
2,5-anhydro aldonic acids, are obtained. Direct nitric acid oxidation of a
hexosamine therefore leads to a 2,5-anhydrosaccharic acid with configura-
tion inverted at carbon 2, since nitrous acid is always present during the
reaction. Isosaccharic acid was so named before its anhydro character was
recognized.

[36] L. von Vargha, Ber., 68, 1377 (1935); L. von Vargha and T. Puskás, Ber., 76,
859 (1943).
[37] P. A. Levene, Biochem. Z., 124, 37 (1921); E. Fischer and coworkers, Ber., 27,
188 (1894); 36, 2588 (1903)

TABLE II

Action of Nitrous Acid on *Hexosamines*

HNO₂ →

Configuration	Hexosamine	Hexosamine Acid	2,5-Anhydro hexose	2,5 Anhydro aldonic Acid	2,5 Anhydro saccharic Acid
D-*ido*	D-idosamine		→	D-idonic	→ D-idaric
D-*gulo*	D-gulosamine		→	D-gulonic	→ D-gularic = L-*gluco* saccharic ↑ (Enantiomorpha)
D-*gluco*	D-glucosamine (chitosamine)	D-glucosamine (chitosamine)	D-glucose epichitose	→ D-gluconic chitaric	→ D-glucaric epicosaccharic
D-*mann o*	D-mannosamine epichitosami	D-mannosamine epichitosami	D-mannose chitose	D-mannonic chitaric	→ D-mannaric (isosaccharic)
D-*galacto*	D-galactosamine chondrosamine	D-galacto-samine chondrosamine	→	D-galactonic epichondronic	→ galactaric
			→	D-talonic chindronic	→ D-talaric

Levene established the configuration of episaccharic acid as 2,5-anhydro-glucaric acid by comparing it with the synthetic enantiomorph, prepared from D-xylose by the cyanohydrin synthesis.

| 2,5-Anhydro-D-idaric acid | levo-Hexosaminic acid | D-Xylose | dextro-Hexos-aminic acid | 2,5-Anhydro-D-gularic (2,5-anhydro-L-glucaric) acid |
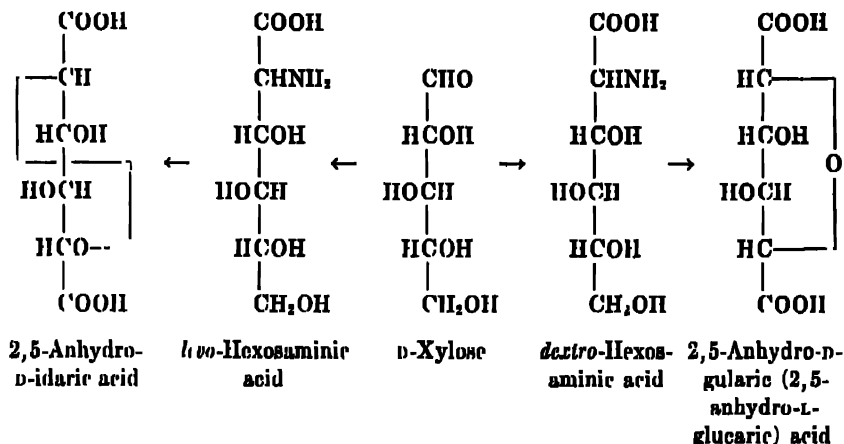
The product of this synthesis can have either the D-*gulo* (L-*gluco*) or D-*ido* configuration whereas isosaccharic and epiisosaccharic acids can have only the D-*manno* or D-*gluco* (L-*gulo*) configuration. Hence, regardless of the configuration of any of the intermediate products, if the dibasic end-product is the enantiomorph of either isosaccharic or epiisosaccharic acid, it must have the L-*gluco* (D-*gulo*) structure. In actual fact, Levene found the following:

	Epiisosaccharic	2,5-Anhydro L *gluco-saccharic* (L-glucaric)
Free Acid	Monohydrate from acetone, m.p. 160°C., water free $[\alpha]_D^{20} + 39.7$ (water)	Crystals from acetone, m.p. 163°C $[\alpha]_D^{20} - 38.8$ (water)
Acid Potassium Salt	$[\alpha]_D + 38.5$ (water)	Monohydrate, $[\alpha]_D^{20} - 38.1$ (water)

Isosaccharic acid melts at 185°C., and has $[\alpha]_D^{20} + 46.1$

From this, epiisosaccharic acid is accorded the 2,5-anhydro-D-*gluco-*saccharic configuration, so that isosaccharic acid is the 2,5-anhydro-D-*manno* isomer.

It remains to be explained, however, why the same anhydro acids results from both epichitosaminic acid and its lactone, whereas in the case of the D-hexosaminic acids prepared from D-xylose, the lactone of the *dextro* 2-epimer and the free acid of the *levo* epimer both give the same 2,5-anhydro-L-saccharic acid. In the case of the hexosaminic acids from xylose,

a Walden inversion must have occurred in the case of either the lactone or the free acid, but not in both. Peat[38] explains the Walden inversion in the chitosamine → chitose reaction as an electronic shift where by, in effect, the pyranose ring oxygen transfers its allegiance from carbon 1 to carbon 2, with accompanying inversion, as a result of diazonium formation
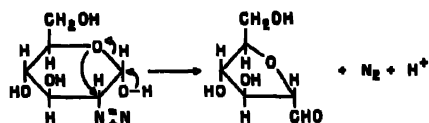
on the amino group at carbon 2. According to this mechanism, the pyranose ring oxygen induces inversion regardless of the configuration at carbon 2.

Chitose and epichitose have not been adequately characterized as 2,5-anhydro aldohexoses. A methyl chitoside has been reported,[39] but chitose is unexplicably resistant to sodium amalgam reduction.[40] (See also, p. 414.)

Hemiacetalization for chitose would be possible only at carbon 4, giving a highly strained 2,5 1,4 structure in equilibrium with the *aldehydo* form, so that the latter should be the more stable modification. Also, it should be remembered that treatment of nonaromatic amino compounds with nitrous acid gives rise not only to the corresponding hydroxy derivative but also to isomeric hydroxy compounds, and to olefins. When ring closure is possible, as between a preexisting hydroxyl or one formed by the deamination, cyclic ethers or olefin oxides can also form. Thus 1,4-diaminobutane yields 1,4-butanediol, 1,3-butanediol, 1-butene-4-ol, 1,3-butadiene, and tetrahydrofuran.[41] 1,4-Diaminocyclohexane gives 4-aminocyclohexanol, cyclohexylamine-3,4-ene, and dihydrobenzene.[42] Monoethanolamine gives acetaldehyde (vinyl alcohol), and 1-aminopropane-2,3-diol forms a reducing compound, believed to be a mixture of dihydroxyacetone and glyceraldehyde; ethylenediamine yields ethylene oxide. Deamination thus resembles in several aspects the hydrolysis of inorganic esters and halohydrins.

D. 1,5-Ether Rings. Only the polyhydric alcohols are represented in this group. (In the sugar series these would be pyranoses and in the acid series δ-lactones.) Styracitol (1,5-D-mannitan)[43] and polygalitol (1,5-D-sorbi-

[38] S. Peat, *Advances in Carbohydrate Chem.*, **2**, 63 (1946).

[39] C. Neuberg, *Ber.*, **35**, 4009 (1902).

[40] R. C. Hockett, Private Communication.

[41] N. Demyanoff, *J. Russ. Phys. Chem. Soc.*, **24**, 348 (1892); *J. Chem. Soc. (Abst.)*, **64**, 453 (1893).

[42] W. A. Noyes and H. H. Ballard, *Ber.*, **27**, 1450 (1894).

[43] Y. Asahina, *Ber.*, **45**, 2366 (1912).

tan)[44] occur naturally in several plant species. They have been synthesized from tetraacetyl-1,2-D-glucoseen by catalytic hydrogenation. Zervas[45] who

```
   CH₂-            CH--            CH₂-
    |               ‖               |
  AcOCH           COAc            HCOAc
    |               |               |
  AcOCH   O       AcOCH   O       AcOCH   O
    |       |       |       |       |       |
  HCOAc   |       HCOAc   |       HCOAc   |
    |               |               |
   HC--- ·         HC   ·          HC
    |               |               |
   CH₂OAc          CH₂OAc          CH₂OAc
```

Tetraacetyl-1,5-D-mannitan   Tetraacetyl-1,2   Tetraacetyl-1,5 D sorbitan
(styracitol)   D-glucoseen   (polygalitol)
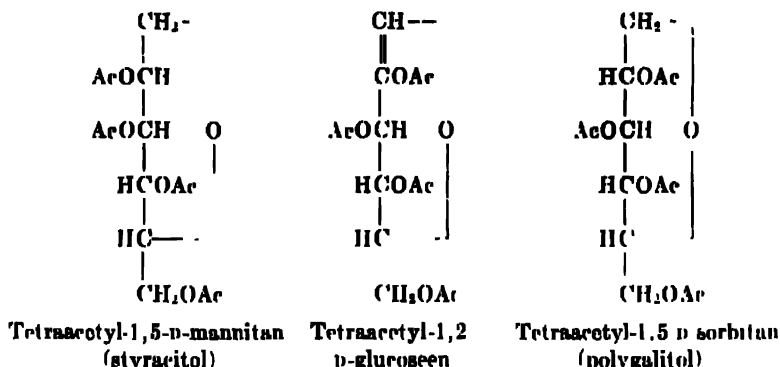
first carried out the synthesis, isolated only the mannitan isomer corresponding to tetraacetylstyracitol. The other predicted 2-epimer, polygalitol, was found in the mother liquors in much smaller amounts by Richtmyer, Carr and Hudson.[46] The synthesis established everything but the configuration at carbon two. Polygalitol was then proved to have the D-*gluco* configuration by deriving it from acetobromoglucose through a series of reactions involving only carbon 1.

A 2-desoxy-1,5-hexitan (hydroglucal) is obtained from the hydrogenation of glucal triacetate and subsequent hydrolysis of the sirupy hydroglucal triacetate to the free hydroglucal.

```
   CH--             CH₂-
    ‖                |
   CH               CH₂
    |                |
  AcOCH   O        HOCH   O
    |       |        |       |
  HCOAc   |   H₂    HCOH   |
    |        ───────→       |
   HC--            deacetylation   HC---
    |                |
   CH₂OAc           CH₂OH
```
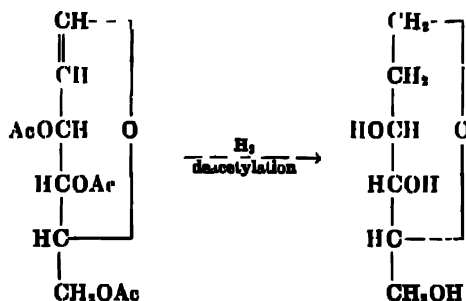
Hydroglucal melts at 86°–87°C., has $[\alpha]_D^{19}$ + 16.4, and is very hygroscopic. Although a second 3,6-ring closure would be possible, the compound is not attacked by boiling concentrated hydrochloric acid. In its solubility behavior, hydroglucal resembles the hexides (dianhydro derivatives) more

[44] J Shinoda, S. Sato and D. Sato, *Ber.*, **65**, 1219 (1932).

[45] L. Zervas, *Ber.*, **63**, 1689 (1930).

[46] N. K Richtmyer, C. J. Carr and C. S. Hudson, *J. Am Chem. Soc*, **65**, 1477 (1943).

than the hexitans (monoanhydro derivatives), since it dissolves readily in acetone, and has some solubility in benzene.[47]

E. Epoxy Derivatives. Anhydride rings formed between contiguous hydroxyl groups are of the ethylene oxide type and as a whole are the most reactive type. Such rings are the usual type formed when tosyloxy groups are removed provided that adjacent free hydroxyls are present and that the groups involved have a *trans* relation. (For a more detailed discussion, see under Tosyl Esters.)

The reactions of 1,2-isopropylidene-5,6-anhydroglucofuranose have been extensively investigated by Ohle and associates. For this substance, the anion adds to carbon 6, and 6-substituted *glucose* ethers are formed; no Walden inversion occurs because this carbon is not asymmetric. The reaction with the sodium alcoholates (RONa) gives the following 6-substituted ether derivatives: 6-methyl, 6-ethyl, 6-propyl, and 6-benzyl-isopropylidene-glucoses.[18] Fusion with phenols leads to the 6-phenyl ether derivatives,[49] and fusion with various amines gives the corresponding amines,[50] e.g., 6-diphenylamino-isopropylideneglucofuranose. The reaction provides a new means of conjugating amino acids with sugars, and, by the reaction with alanine ester followed by acid hydrolysis, 6-N-alanino-D-glucose is obtained.[51] The preparation of 6-acylglucoses is carried out by heating the 1,2-isopropylidene-5,6-anhydro-D-glucofuranose with carboxylic acids in the presence of a small amount of pyridine; the 6-thioalkyl derivatives are produced by the action of mercaptans or hydrogen sulfide dissolved in barium hydroxide.[52] Other anhydro sugars of this type have not been as well studied but appear to react in analogous fashion The reaction with ammonia, leading to amino sugars, is of particular importance for the preparation and study of the configuration of the amino sugars (see Chapter IX).

When the anhydro ring connects two asymmetric carbons, cleavage of the ring may take place at either of the bonds (C' –) –(C') with inversion, and two products are generally formed. For instance, the cleavage of the ethylene-oxide ring of methyl 2,3-anhydro-α-D-alloside by ammonia may take place with inversion at carbon 2 to give methyl 2-amino-α-D-altroside or at carbon 3 to give methyl 3-amino-α-D-glucoside.[53] (The reaction was carried out for the 4,6-benzylidene derivatives.) Isbell[54] explains the reac-

[47] E Fischer, *Ber*., *47*, 196 (1914); M. Bergmann and W Freudenberg, *ibid*, *62*, 2783 (1929)

[18] H Ohle and K Tessmar, *Ber*, *71*, 1843 (1938)

[49] H Ohle, E Euler and R Voullième, *Ber.*, *71*, 2250 (1938)

[50] H. Ohle *et al.*, *Ber.*, *71*, 27 (1938); *69*, 1636 (1936)

[51] B Helferich and R. Mittag, *Ber.*, *71*, 1585 (1938).

[52] H. Ohle and W. Mertens, *Ber.*, *68*, 2176 (1935).

[53] W. Lake and S. Peat, *J. Chem. Soc.*, 1417 (1938).

[54] H S Isbell, *Ann. Rev. Biochem.*, *9*, 65 (1940).

tion as follows: the negative substituent group (NH₂) approaches carbon 2 or carbon 3 from a direction opposite to the position of the oxygen forming

the anhydro ring; as the new bond is being made with the carbon, the oxygen of the anhydro ring is released and combines with a proton forming the hydroxyl which remains with the other carbon originally a member of the ring.

F. Biochemistry and Reactions of Sugar Alcohol Anhydrides (Glykitans). Attention has already been drawn to the practical analogy between the glycoses and the glykitols with respect to cyclization during reaction. The glykitols are, of course, much more stable towards alkalies and acids than the common sugars, and yield the expected substitution derivatives when alkylated, acetylated, nitrated or acetalated by customary procedures. However, when heated with organic acids, with or without additional catalyst, at the temperature of 180° to 250° customary in industrial ester or resin synthesis, the hexitols undergo anhydridization. Anhydridization is likewise effected by heating with catalytic amounts (less than 1%) of strong mineral acids, or by other dehydrating agents.[55] These anhydrides have been used as such, in the form of non-crystallizing mixtures, as softeners for paper and textiles,[56] and humectants, particularly for tobacco.[57] The synthesis of individual hexitans and certain hexides has already been discussed.

a. BIOCHEMISTRY[58]

Except in massive doses, no toxicity on feeding has been found for any of the known mono- or dianhydrides of the hexitols. Isomannide is secreted unchanged, and without any toxic symptoms, up to at least 20 g. in man. It has been patented as a diuretic.[59] Arlitan resembles inulin, the hexitols and creatinine in being excreted by the kidney without reabsorption. Isosorbide and isomannide differ, however. A total of 80 g. of arlitan has been injected within 2 hours into humans without apparent toxic symptoms.[60] None of the anhydrides, including hydroglucal,[61] with the possible

[55] J. Muller and U. Hoffmann, U. S. Patent 1,757,468, May 6, 1930.
[56] British Patent 294,130, March 16, 1927.
[57] R. M. Goepp, U. S. Patent 2,371,389, Mar. 13, 1945
[58] See: C. J. Carr and J. C. Krantz, *Advances in Carbohydrate Chem.*, 1, 180 (1945).
[59] J. C. Krantz, U. S. Patent 2,143,324, Jan. 10, 1939.
[60] W. W. Smith, N. Finkelstein and H. W. Smith, *J. Biol. Chem.*, 135, 231 (1940).
[61] W. Freudenberg and G. E. Felton, *J. Biol. Chem.*, 99, 647, 657 (1933).

exception of styracitol, are metabolized by mammals to an appreciable extent. Some styracitol is stored as glycogen when fed with fat.[62]

Polygalitol and styracitol are fermented less readily than their parent hexitols, but more readily than ethylene or propylene glycol, by bacteria of the colon-aerogenes group. These bacteria do not attack ethylene or propylene oxide, erythritol or erythritan, glycidol, or pentaerythritol.[55]

With regard to hemolysis rates of red corpuscles, as measured with dog's blood by the method of Jacobs,[63] it is interesting that the hexitans act slowly, and the rates are intermediate between those of erythritol (77 minutes) and sorbitol (150 minutes), the fastest acting of the three common hexitols. Isosorbide and isomannide are much faster (23 and 8 secs.), and comparable with the rapid ethylene glycol, ethylene oxide, glycerol, glycidol, erythritan and methanol. The effect is osmotic, though not in accordance with the molecular weight, and is repressed by isotonic salt solutions. This huge difference between hemolysis rates for isomannide (6-16 secs.) and mannitol (>4 hours) is true of eight different species of mammals.[64]

Polygalitol, the closest relative to ordinary glucose, is the only hexitan with a sweet taste; it lies between mannitol and sucrose in threshhold sweetness. Erythritan is likewise sweet in low concentration.[65] The other monoanhydrides have a definite astringency, and for isomannide and isosorbide the taste is predominantly bitter. Hydroglucal is faintly sweet.

b. REACTIONS WITH INORGANIC REAGENTS

The effect of the anhydrides on the dissociation of boric acid is not consistent. Erythritan, with a *cis* glycol pair in a 5-membered ring, has the strongest potentiating effect of any known substance, yet 1,4-mannitan, with a very similar configuration, is less effective than the open-chain mannitol. Polygalitol, with no *cis* hydroxyls in a 6-ring, is ineffective, but so is *meso*-inositol, with three *cis* hydroxyls in the same size ring.[66]

The hydrolysis of galactitol 1,6-dichlorhydrin or dibromohydrin with hot water leads to the corresponding crystalline galactitan monohalohydrins and eventually to a noncrystalline galactitan. Strong hydrochloric or hydrobromic acid regenerates the galactitol 1,6-dihalohydrin from the galactitan monohalohydrin. With the latter, alcoholic ammonia yields an aminogalactitol, $C_5H_8(OH)_5NH_2$. Sodium amalgam reduction of the galactitan di- or mono-chlorohydrin likewise yields galactitan.[67]

[62] C. J. Carr and S. E. Forman, *J. Biol. Chem.*, **128**, 425 (1939).
[63] M H. Jacobs and A. K. Parpart, *Biol. Bull.*, **60**, 95 (1931).
[64] A. M. Kunkel, C. J Carr, J. C Krantz, Jr., *Proc. Soc. Exptl. Biol Med.*, **42**, 438 (1939).
[65] C. J. Carr and J. C. Krantz, Jr., *J. Am. Pharm. Assoc.*, **27**, 318 (1938).
[66] J. C. Krantz, Jr., C. J Carr and F. F Bock, *J. Phys. Chem.*, **40**, 927 (1936).
[67] G. Bouchardat, *Ann. chim. phys.*, [4] **27**, 145, 184 (1872).

With mannitol and its halohydrins, the reactions are analogous, except that the mannitan monohalohydrin products are less well characterized.[68]

The well-characterized isomannide (1,4-3,6-mannide) can be converted to the 2,5-dichlorohydrin by treatment with phosphorus pentachloride, or thionyl chloride and pyridine. This dichlorohydrin, m.p 67°, is exceptionally stable, being unattacked by fused caustic potash, phosphorus pentachloride at 125°, sodium amalgam, zinc and dilute sulfuric acid. Fuming hydrochloric acid, however, opens the rings to form crystalline mannitol 1,2,5,6-tetrachlorohydrin, m.p. 70°.[69]
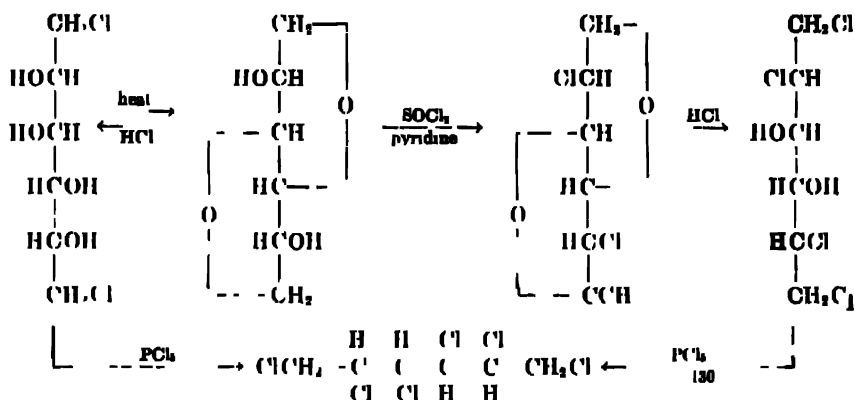
Hydriodic acid opens the ring of styracitol and forms the same secondary hexyl iodide as is obtained from mannitol itself.[70]

Although galactides are not formed from galactitol and hydrohalic acid, phosphoric acid in excess and galactitol give at 135°C. under vacuum a phosphoric ester of a 1,5 3,6-galactide, characterized as its dibenzoate, of m.p. 138°. Studies of models indicate that galactitol cannot form a 1,4-3,6-dianhydride.[71] Mannitol, however, gives isomannide and the phosphate esters thereof under these conditions.[72]

Similarly, whereas the sodium amalgam reduction of mannitol 1,6-dichlorohydrin yields the as yet unidentified "β-mannide," from dulcitol 1,6-dichlorohydrin only a sirupy monoanhydride is obtained, and not a dulcide [74]

With cold, mixed nitric and sulfuric acids, styracitol gives an explosive tetranitrate, m.p. 106°C., $[\alpha]_D^{17}$ −31.82.[71] With the same reagents, iso-

[68] G Bouchardat, Ann. chim. phys., [5] 6, 113 (1875).

[69] See· L. F. Wiggins, J Chem. Soc., 4 (1945).

[70] Y. Asahina, Arch Pharm., 245, 325 (1907).

[71] P. Carré, Compt. rend , 139, 637 (1904); H. G. Fletcher, Private Communication.

[72] P. Carré, Ann. chim. phys., [8] 5, 345-432 (1905).

[73] See: A. Siwoloboff, Ann., 233, 368 (1886).

[74] Y. Asahina, Arch. Pharm., 247, 157 (1909).

sorbide and isomannide give crystalline dinitrates, melting at 52° and at
65.5°, whereas 1,4-mannitan and 1,4-sorbitan yield only sirupy products.
These anhydride nitrates show depressor action, similar to that of glycerol
trinitrate and mannitol hexanitrate.[75] Dilute nitric acid oxidizes the mono-
anhydrides to a mixture of lower acids, chiefly oxalic.

Oxidation of either polygalitol or styracitol with hydrogen peroxide and
ferrous sulfate leads to a product from which glucosazone can be isolated.
With hypobromite, however, the original ring is apparently not attacked,
but instead the 3-osone of 1,5-anhydrofructose is obtained, identified as
the corresponding osazone.[76]

Anhydro osazone, m. p. 184°C.
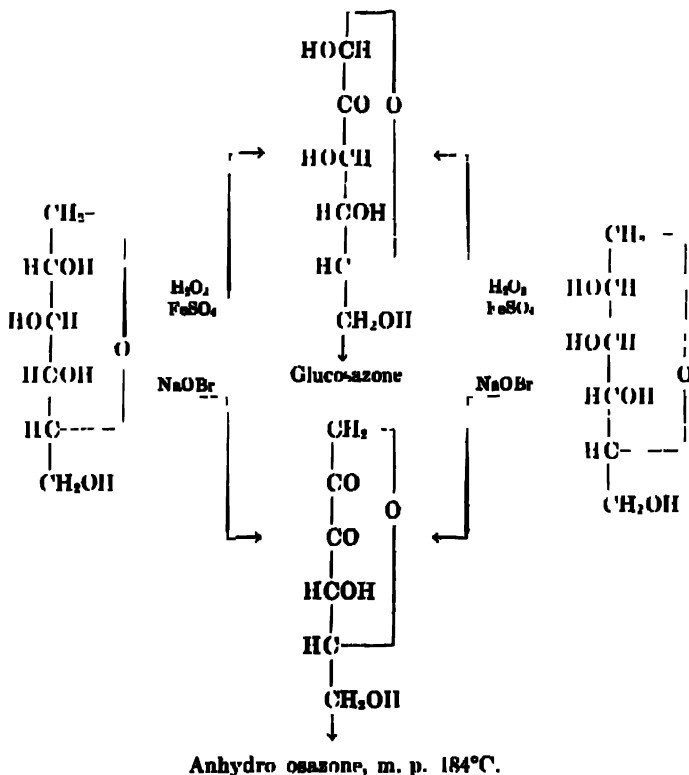
Lead tetraacetate cleavage of the hexitans proceeds normally. The 2,5-
sorbitan takes up one mole of oxidant very slowly. Both 1,4-mannitan and
3,6-sorbitan, each with a *cis* glycol pair in a five-membered ring, show
rapid consumptions of one mole of oxidant, followed by a slower uptake.
1,4-Sorbitan and 3,6-galactitan, with a *trans* ring glycol pair, show a

[75] S. E. Forman, C. J. Carr and J. C. Krantz, Jr., *J. Am. Pharm. Assoc.*, **30**, 132
(1941); J. C. Krantz, Jr., and coworkers, *J. Pharmacol.*, **67**, 187, 191 (1939).
    [76] See: J. Shinoda, S. Sato and D. Sato, *Ber.*, **65**, 1219 (1932).

gradual uptake of more than two equivalents, the external glycol pair being oxidized more slowly than the ring pair. Styracitol, with one *cis* ring glycol pair shows a definitely faster uptake than the completely *trans* polygalitol, but the difference in oxidation rates is markedly less than in the five-membered series. This may be due to the limited rotation permitted ring hydroxyl groups in six-membered rings, compared with the virtually fixed orientation of the furanoid rings.[77]

c. REACTIONS WITH ORGANIC REAGENTS

The glycol pairs in erythritan or 1,4-mannitan can be removed to give the corresponding unsaturated derivatives, by treatment with formic acid. Erythritan gives *sym.* dihydrofuran,[78] and 1,4-mannitan, under similar treatment, yields in addition to isomannide a strongly levorotatory vinyl-dihydrofuran, b.p. 107°-109°, $[\alpha]_D$ −168°.[79] van Romburgh and van der Burg proved the vinyldihydrofuran structure by hydrogenation over colloidal palladium to the inactive α-ethyltetrahydrofuran, and establishment of the identity of this ethyltetrahydrofuran with that obtained by the different route from Wohlgemuth's 1,4-hexanediol:
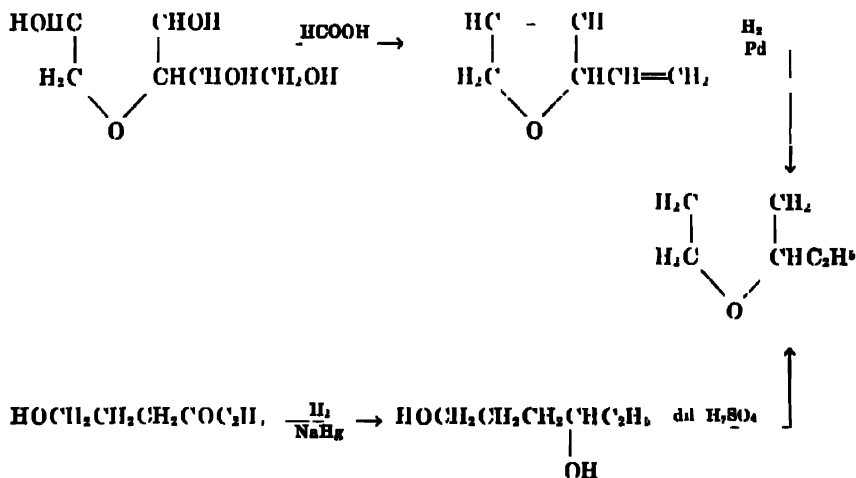
In view of the close similarity in physical constants between the α-ethyltetrahydrofuran and the isomeric dimethyltetrahydropyran, the identity was established by splitting the ring with hydrobromic acid to the 1,4-dibromohexane, and converting this to the quarternary ammonium salt

[77] *cf.* R. C. Hockett, M. T. Dienes and H. E. Ramsden, *J. Am. Chem. Soc.*, **65**, 1474 (1943).
[78] A. Henninger, *Ann. chim. phys.*, [6] 7, 211, 217 (1886).
[79] A. Fauconnier, *Compt. rend.*, *100*, 914 (1885); *cf.* A. Henninger, *Ber.*, 7, 264 (1874).

with piperidine.[80] Since carbon 5 of α-ethyltetrahydrofuran is asymmetric, the hydrogenation must have caused racemization.

Isomannide diformate decomposes on heating to regenerate isomannide, with loss of carbon monoxide.[81] This behavior, consistent with absence of vicinal hydroxyls, parallels the thermal decomposition of pentaerythritol tetraformate to give pentaerythritol and pure carbon monoxide.[82] Hurd and Filachione obtained from the formic acid reduction of mannitol, in addition to 2-vinyl-2,5-dihydrofuran, a monoformate derivative to which they ascribed the formula:[83]

$$HC - CH$$
$$H_2C \quad CHCHOHCH_2OCHO$$
$$O$$
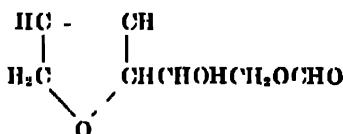
The secondary hydroxyl groups in isomannide and isosorbide can be tosylated,[84] and both tosyloxy groups of isomannide are replaced by iodine by heating with sodium iodide in acetone for five hours. In the case of isosorbide only one tosyloxy group is replaced under these conditions. The configuration of these mono- and di-iodohydrins is not known, since Walden inversion may take place during their preparation.

Bloor attempted to esterify mannitol with lauric acid in concentrated sulfuric acid solution at 38°C., and obtained a mannitan dilaurate having the unusually high melting point of 122°, and convertible on heating at 200°C. to a dianhydromannitol dilaurate, m.p. 37.5°C., $[\alpha]_D$ + 125°. The high rotation and the method of synthesis argue for the isomannide structure assigned by Bloor. The corresponding reaction with stearic acid, at 70°C., gave a mannitan distearate, m.p. 124°, $[\alpha]_D^{50}$ + 8.0, and a mannide distearate, m.p. 51°, $[\alpha]_D^{20}$ + 64.8, which yielded mannitol when saponified by alcoholic sodium hydroxide.[85] The mannitan distearate when heated to 200°C. gave a dianhydromannitol distearate of m.p. 61.5°, $[\alpha]_D^{20}$ + 93.7, probably an isomannide derivative.

The alcoholysis of mannitol with tristearin, using sodium ethylate catalyst, at 220°-250°, yields a crystalline material of m.p. 67°-72°, but

[80] P. van Romburgh and J. H. N van der Burg, *Proc. Acad. Sci. Amsterdam*, **25**, 335 (1922).

[81] A. Fauconnier, *Bull. soc. chim* , **41**, 18 (1884)

[82] P. van Romburgh, *Proc .Acad. Sci. Amsterdam*, **10**, 166 (1907).

[83] C. D. Hurd and E. M. Filachione, *J Am. Chem Soc.*, **61**, 1156 (1939).

[84] R. C. Hockett, H. G. Fletcher, E. L. Sheffield, R. M. Goepp and S. Soltzberg, *J. Am. Chem. Soc.*, **68**, 930 (1946).

[85] W. R. Bloor, *J. Biol. Chem.*, **11**, 141, 429 (1912); **7**, 427 (1910). Berthelot made a sirupy dianhydromannitol, which he called mannide, by heating mannitol and butyric acid at 200–250°. This material was *partly* converted back to mannitol by long standing in air.

with a rotation of −1.44°. This product, described from the analysis as a mixture of mannitan distearate and isomannide distearate cannot be a binary mixture of Bloor's mannitan distearate and mannide distearate in view of the negative rotation.[86] The corresponding alcoholysis with olive oil yields an oil, analyzing for a similar mixture of mono and dianhydro diester.[87]

Of considerable industrial importance are the ester products obtained by the direct heating of hexitols with one or more equivalents of the fatty acids derived from hard fats, drying oils and semidrying oils. Acid or alkaline catalysts, and temperatures of 200–250° are used, with vigorous agitation and, usually, a current of carbon dioxide. The partial esters, in particular, have valuable surface-active properties,[88] and their comparatively bland taste and practical freedom from toxicity[89] give them a wide field of usefulness as emulsifiers, solubilizing and blending agents. Products of this type were first synthesized by Berthelot, by heating the reactants in a sealed tube. Long and co-workers demonstrated a commercially practicable synthesis by vigorous stirring in presence of an inert gas.[89] Products with similar properties have been made from glycerol and other polyols. The manufacture and uses of these fatty esters have been comprehensively reviewed.[90] The partial ester derivatives from the hexitols rank with the sorbose fermentation of sorbitol for ascorbic acid synthesis, and explosive and drug use of mannitol hexanitrate as major industrial outlets for the hexitols.

In the trade, the partial esters go by the nominal designation, e.g., mannitan monooleate, or by trade names. Span 20, 40, 60 and 80 are sorbitan monolaurate, palmitate, stearate and oleate, respectively. These products are dispersible in water but not soluble. An equally useful series of water-soluble surface-active products are obtained from the above partial esters by reaction with ethylene oxide to give polyglycol ethers (Tweens). From ten to thirty moles of ethylene oxide per mole of fatty acid are required for satisfactory water solubility, depending on the chain length of the acid. Since the water solubility is conferred by a plurality of neutral nonionic oxyethylene groups, $- OC_2H_4(OC_2H_4)_n -OC_2H_4OH$, the products are not electrolytes in the ordinary sense like the anionic or cationic soaps and do not lose their effectiveness in solutions containing strong electrolytes.

Other than that they are mixtures of esterified hexitol anhydrides, little is known about the actual composition of these esterification products.

[86] A. Lapworth and L. K. Pearson, *Biochem. J.*, *13*, 296 (1919)

[87] W. D. Halliburton, J. C. Drummond and R. K. Cannon, *Biochem. J.*, *13*, 301 (1919).

[88] First recognized, for products from mannitol, by G. Isar and P. Ferro, *Biochem. Z.*, *59*, 234 (1914).

[89] J. S. Long, W. W. Kittelberger, L. K. Scott, and W. S. Eggs, *Ind. Eng. Chem.*, *31*, 953 (1939).

[90] H. A. Goldsmith, *Chem. Rev.*, *33*, 257–349 (1943).

There is evidence that their usefulness depends in part on their mixed character, since a relatively pure sorbitan monolaurate, synthesized from 1,4-sorbitan and lauroyl chloride, is much inferior in solubility characteristics and emulsifying power to the technical product derived from sorbitol and commercial lauric acid (containing minor amounts of C_{10}, C_{14} and C_{18} acids) and shows somewhat less than the required hydroxyl content for a hexitan monoester.

Synthetic drying oils from sorbitol[91] or mannitol[92] and drying oil acids have been described.[91] The full esters of hexides and saturated medium length fatty acids are useful as plasticizers.[93] Analogous esterification products have been obtained from the reaction of hexitols with rosin drying oil acids,[94] phthalic anhydride,[95] succinic and citric acid.

3. Unsaturated Sugars (Glycals and Glycoseens)

Two types of unsaturated sugar derivatives are known which may be considered to be derived from the corresponding sugar by removal of two hydroxyl groups (glycals) or a molecule of water (glycoseens).
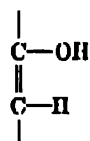
Glycoseen
double bond
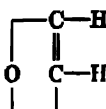
Glycal
double bond

Products of still higher degrees of dehydration are prepared by the action of acids, acetoacetic ester or formic acid on carbohydrates (see p. 69 and 231). Furan and pyran derivatives or levulinic acid are the principal products of this type. Under alkaline conditions, enediols are in equilibrium with reducing sugars. Ascorbic acid has an enol system, and lactones of *manno*-saccharic acid are converted to derivatives of this type upon treatment with alkali.

A. Glycals. The glycals, first reported by Fischer,[96] were extensively investigated by Bergmann and Schotte and are important intermediates

[91] J. D. Brandner, R. H. Hunter, M. D. Brewster, R. E. Bonner, *Ind. Eng. Chem.*, 37, 809 (1945).

[92] A. A. Blagonravova and A. Y. Drinberg, *J. Applied Chem.* (U.S.S.R.), 11, 1642 (1938); *Chem. Abst.*, 33, 5805 (1939).

[93] R. M. Goepp, Jr., U. S. Patent 2,394,439, Feb. 5, 1946.

[94] E. Schaal, U. S. Patent 335,485, Oct. 11, 1884, Re. 10,823; Ger. Patent 500,504, July 11, 1925.

[95] R. H. Kienle, *Ind. Eng. Chem.*, 22, 593 (1930), R. M. Goepp, Jr., and K. R. Brown, *ibid.*, 30, 1222 (1938); British Patent 350,992, Dec. 16, 1029; 322,537, June 2, 1928; Fr. Patent 703,792, Oct. 17, 1930.

[96] E. Fischer, *Ber.*, 47, 196 (1914).

for the synthesis of sugars from their 2-epimers (see Chapter III). They result from the reduction of the acetylglycosyl bromides with zinc and

$$
\begin{array}{l}
\left[\begin{array}{l} HCBr \\ HCOAc \\ AcOCH \end{array} O \right]
\quad \xrightarrow[HOAc]{Zn}
\quad \left[\begin{array}{l} HC \\ HC \\ AcOCH \end{array} O \right]
\quad \xrightarrow{OH^-}
\quad \left[\begin{array}{l} HC \\ HC \\ HOCH \end{array} O \right]
\end{array}
$$

Tetraacetyl-   Triacetyl-   Glucal
glucosyl bromide   glucal
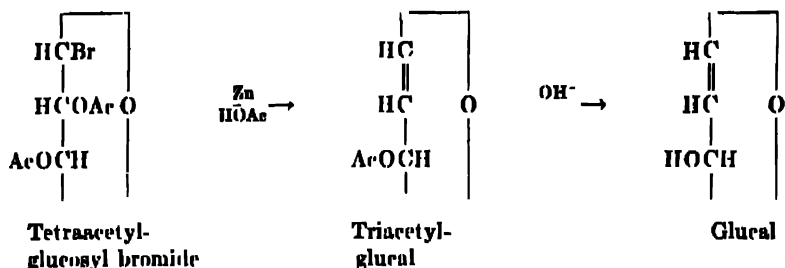
acetic acid. The structure of glucal is established by the following evidence. The presence of a double bond is shown by the addition of bromine and chlorine to give a dihalide and by the oxidation with ozone to D-arabinose.[97] Methylation of glucal gives a trimethyl derivative which upon oxidation with perbenzoic acid gives 3,1,6-trimethylglucose.[98] Inasmuch as carbons 1 and 2 have lost their asymmetry, alpha and beta isomers of glycals are not possible, and sugars epimeric at carbon 2 gives the same glycal.

Glucal reduces Fehling solution and is stable under weakly alkaline conditions but rapidly decomposes in the presence of acids to give green-colored solutions. A pine splinter soaked in glucal solution turns intensively green when held in hydrochloric acid vapor. One of the most important reactions is the oxidation by perbenzoic acid. This acid, $C_6H_5CO(O_2)H$, adds two hydroxyls to ordinary ethylenic double-bonds with the formation of glycols and to glycals with the production of sugars.[99] Since carbon 2 becomes asymmetric, two epimeric sugars are produced, the proportions varying according to the glycal and to the presence of substituent groups.[100] Glucal and rhamnal yield mannose and rhamnose, respectively, as the main products, but galactal yields considerable quantities of both galactose and talose. For the acetylated and methylated glycals, quite different yields are often obtained, e.g., trimethyl- and triacetyl-glucal give glucose derivatives principally rather than mannose derivatives. It seems probable that the 1,2-anhydro sugars may be intermediates in the reaction as the 5,6-anhydro sugars are known to react with carboxylic acids in a similar fashion yielding 6-acylsugars (see p. 361). Such a mechanism is also indicated by the presence of monobenzoyl sugars among the reaction products

[97] E. Fischer, M. Bergmann and H. Schotte, Ber., 53, 509 (1920)

[98] E. L. Hirst and C S Woolvin, J. Chem. Soc , 1131 (1931)

[99] M. Bergmann and H. Schotte, Ber., 54, 440, 1564 (1921).

[100] P. A. Levene and A. L. Raymond, J. Biol. Chem., 88, 513 (1930); P. A. Levene and R. S. Tipson, ibid., 93, 631 (1931).

of galactal and perbenzoic acid.[101] In anhydrous alcohols, glycosides are synthesized from the glycals.[99]

Methyl α-mannoside   D Glucal   Probable intermediate

D-Arabinose   Glucose (and mannose)
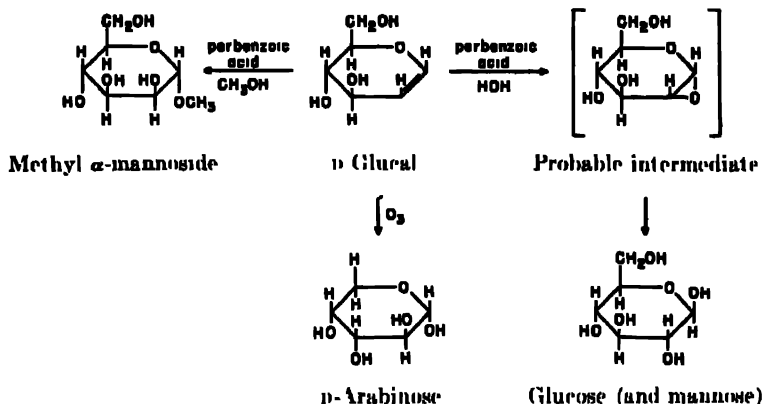
Treatment of the glycals with cold dilute sulfuric acid gives sulfuric esters which, after hydrolysis, yield 2-desoxysugars (see Chapter III). Chlorine adds to the double bond with the production of epimeric 1,2-dichloro derivatives, whereas hydrobromic acid appears to give 2-bromo derivatives. Oxidation of the glycals with ozone splits the molecule at the double bond, and reduction with hydrogen in the presence of a catalyst yields hydroglycals.

The tendency of the glycals to undergo intramolecular change requires particular mention. The heating of an aqueous solution of triacetylglucal causes the migration of the double bond to the 2,3 position and the hydrolysis of one acetyl group.[102] The product, diacetylpseudoglucal, on treatment with barium hydroxide undergoes further rearrangement to give isoglucal and protoglucal.[103]

Triacetylglucal   Diacetylpseudoglucal   Isoglucal   Protoglucal
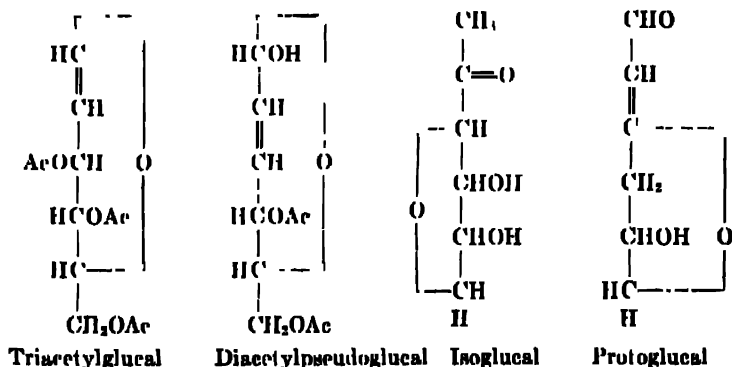
[101] W. W. Pigman and H. S. Isbell, *J. Research Natl. Bur. Standards, 19*, 189 (1937).

[102] M. Bergmann and W. Freudenberg, *Ber., 64*, 158 (1931).

[103] M. Bergmann, L. Zervas and J. Engler. *Ann., 508*, 25 (1934)

B. Glycoseens. Whereas the double bond of the glycals is formed by the elimination of a bromine atom and an acetyl group (equivalent to two hydroxyl groups), the removal of the elements of hydrobromic acid from the acetylglycosyl halides leads to glycoseens (oxyglycals).[104] The reaction, carried out by heating (60°C.) the halide with a secondary amine, is some-
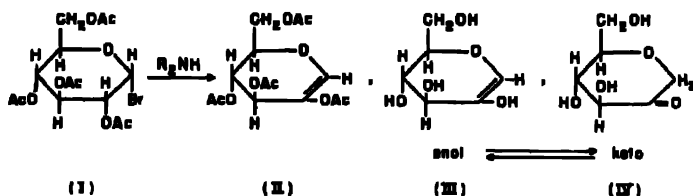
(I)   (II)   (III)   (IV)

what similar to the Hofmann degradation of quaternary amines. As illustrated, the 1,2-glucoseen (the enol isomer-III) is probably in equilibrium with the keto isomer (IV). Only the acetylated enol form (II) has been crystallized, and removal of the acetyl groups probably allows isomerization to take place. The deacetylated product has not been crystallized, and on acetylation does not revert to the original crystalline material. Two phenylhydrazine groups are introduced into the molecule when the acetylated isomer (II) and phenylhydrazine interact. This product is apparently an osazone type of derivative of the keto isomer.[105]

Kojic acid, which is formed by the action of many fungi and bacteria on carbohydrates (sugars, inulin, dulcitol, glycerol, etc.), may be synthesized from glucose,[106] and, since it contains no asymmetric carbons, from any other hexose. Starting with the tetraacetyl-1,2-glucoseen (V), the synthesis is carried through by reaction with chlorine to give the 1,2-dichloro derivative (VI) from which the chlorine is replaced by the aid of moist silver carbonate with the formation of the acetylated hemiacetate of glucosone hydrate (VII). This is converted directly to the diacetylkojic acid (VIII) by the action of acetic anhydride and sodium acetate.

The naturally occurring anhydrohexitol styracitol has been synthesized[107] by hydrogenation and deacetylation of tetraacetyl-1,2-glucoseen (V).

The 5,6-glycoseen derivatives (X) have been investigated by Helferich and associates.[108] They are prepared by treatment of the 6-bromo or 6-iodo sugar derivatives (IX) in pyridine solution with silver fluoride or silver sulfate. The hydrolysis of 1,2-3,5-diisopropylidene-6-tosylglucose with ammonia or sodium methylate leads to 1,2-3,5-diisopropylidene-5,6-

[104] K. Maurer, Ber., 62, 332 (1929)

[105] M. Bergmann and L. Zervas, Ber , 64, 2032, 1434 (1931)

[106] K. Maurer and A. Muller, Ber., 63, 2069 (1930); K. Maurer, ibid., 63, 25 (1930)

[107] L. Zervas, Ber., 63, 1689 (1930).

[108] B. Helferich and E. Himmen, Ber., 61, 1825 (1928).

```
   ┌─────────┐                    Cl
   CH        │              Cl₂   CH────────┐                  OH
   ‖         │              ──→    │         │          Ag₂CO₃    │
   COAc      │O                   (Cl)C(OAc) │O         ─────→    CH────────┐
   │         │                     │         │          HOH      (HO)C(OAc) │O
AcOCH        │                  AcOCH        │                    │         │
   │         │                     │         │                 AcOCH        │
  (V)                              (VI)                           │         │
                                                               HCOAc        │
                                                                  │         │
                                                               HC───────────┘
                                                                  │
                                                               CH₂OAc
                                                                 (VII)
```

```
   CH────────┐
   ‖         │
   COAc      │       (Ac)₂O
   │         │O     ───────→
   C═O       │       NaOAc
   │         │
   CH        │
   ‖         │
   C─────────┘
   │
  CH₂OAc
   (VIII)
```
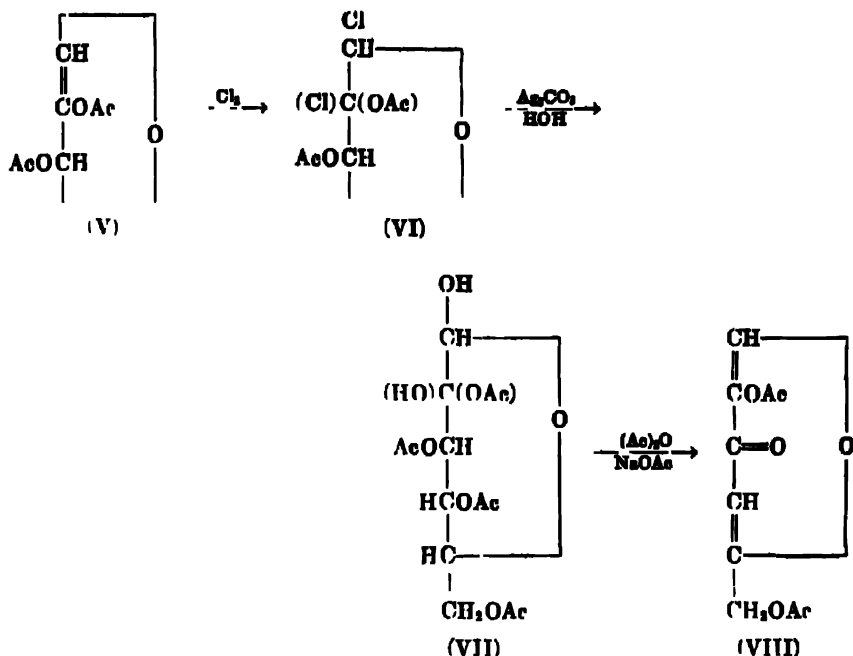
glucoseen and other products.[109] When isomerization to the keto isomer is made impossible by involvement of the hydroxyl of carbon 5 in the stable pyranose ring of a glycoside or an isopropylidene ring, the enol derivative (X) frequently is crystallizable. Methyl 5,6-glucoseenide has been obtained by the Helferich and Himmen method. When the ring is labilized by acid hydrolysis of the glucoside, conversion to the keto isomer (XI), 5-keto-6-desoxyglucose, takes place. The corresponding acetone derivatives undergo similar transformations.[110]

```
   │                     │   │                  │
   C─ ─     │      AgF   ─C─      O      H⁺    ─C─
   ‖        │O    ──→     │       │     ──→     │
   HC       │             C───────┘            C═O
   │        │             ‖                     │
   H₂C                    CH₂                   CH₃
   (IX)                   (X)                   (XI)
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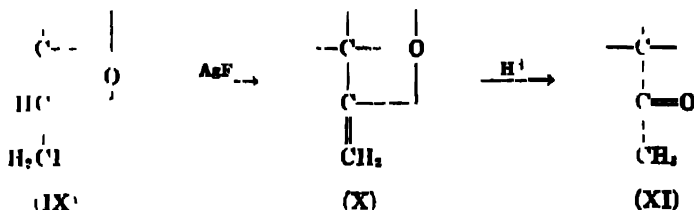
At one time, these derivatives were of considerable interest from the standpoint of the structures of the glycosides.[111] The furanoid 5,6-glyco-

[109] H. Ohle and L. v. Vargha, Ber., 62, 2425 (1929).

[110] B. Helferich and E. Himmen, Ber., 63, 2136 (1920); H. Ohle and R. Deplanque, ibid., 66, 12 (1933)

[111] H. Bredereck, Ber., 63, 959 (1930); A. Müller, ibid., 65, 1051 (1932).

seenides would be expected immediately to isomerize to the keto isomers while the corresponding derivatives with pyranoside rings cannot isomerize. The product obtained from the furanosides would be a ketone of the general structure R—CO—CH$_3$. A compound of this structure would give the iodoform reaction, and a lack of this reaction would be indicative of a pyranose structure. The evidence obtained by this method agrees well with other methods, but the formation of the 5,6-glycoseens is apparently not a general reaction for furanosides.[113]

[113] B. Helferich and O. Lang, *J. prakt. Chem.*, [2] *133*, 321 (1932)

NITROGENOUS DERIVATIVES

(N-GLYCOSIDES, NUCLEIC ACIDS AND HYDROLYSIS PRODUCTS, HYDRAZONES, OSAZONES, OXIMES, AMINO SUGARS, ETC.)

Nitrogenous carbohydrate derivatives such as the nucleic acids, nucleoproteins, some viruses, several vitamins of the B-complex, and some coenzymes are undoubtedly to be considered among the most important of the carbohydrate derivatives. Many of the polysaccharides which exhibit highly specific and characteristic immunological reactions yield amino sugars after hydrolysis. Other sugar derivatives containing nitrogen have considerable importance for purposes of identification and synthesis. The ease with which the sugars react with amines, amino acids and proteins makes it probable that the resulting derivatives are of greater biological importance than has been generally realized. These derivatives may have an important role in the changes of solubility and of color that take place during the drying of foods (melanoidin reaction).

The most common type of nitrogenous derivatives is that which is formed by the reaction of the aldehyde (or hemiacetal) group of the sugars with compounds containing amino groups.

$$R \quad CHO + R'NH_2 \rightarrow R \quad CH-NR' + H_2O$$

If this equation represents the reaction, the products are Schiff bases, but it is possible that the ring form of the sugar reacts:

$$
\begin{array}{c}
HCOH \\
| \\
HCOH \\
|
\end{array} \Bigg] O
+ R'NH_2 \rightarrow
\begin{array}{c}
HCNHR' \\
| \\
HCOH \\
|
\end{array} \Bigg] O
+ H_2O
$$
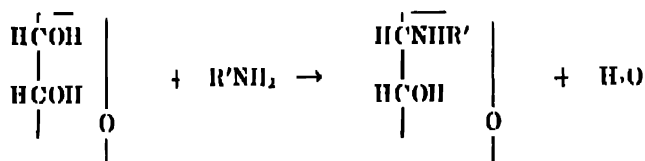
The compounds represented by $R'NH_2$ include alkyl and aryl amines, hydrazines, oximes, ammonia and amino acids. Usually amide groups will not condense readily in this fashion, but urea derivatives are known.

Hydrocyanic acid adds readily to sugars. As this reaction has its primary use in the synthesis of the higher sugars, it is discussed in another chapter (Chapter III).

An important additional group of nitrogenous derivatives is the amino sugars among which are the glycosamines. These compounds represent sugars in which the hydroxyl of a primary or secondary alcohol group has been replaced by an amino group. Several are naturally occurring.

Although many derivatives have been made by condensing sugars with substances containing NH_2-groups, the chemistry of the compounds is still in a very incomplete state. Many of the compounds in which the hemiacetal hydroxyl group of the sugar is substituted by a N-R or a similar group mutarotate when dissolved in solution. Evidently, these compounds are much less stable than the corresponding glycosides. The mutarotations appear to arise from numerous causes which include (1) dissociation into the sugar and nitrogenous base, (2) isomerization between the various ring and open chain forms, and (3) structural changes such as the rearrangement of aldose to ketose derivatives. Many of these compounds are known to exist in both the ring and acyclic forms. Very little is known concerning the relationship of the strength of the base to the stability and the properties of its condensation products with the sugars.

1. Glycosylamines

A. Osimines or Primary Glycosylamines. The treatment of sugars in alcoholic solution (or suspension) with ammonia produces glycosylamines (glycosimines or glycose ammonias) by the replacement of one hydrogen of NH_3 by a glycosyl group.[1] The same reaction takes place readily in liquid ammonia solution. This class of substances is comprised of the lowest homologs of the N-glycoside series. Sometimes two hydrogens are replaced by glycosyl groups with the formation of disaccharide-like substances. The osimines may have a cyclic structure with a free amino group or have an imine structure analogous to that of the hydrazones and Schiff bases.

$$
\begin{array}{ccc}
\begin{array}{l} HCNH_2 \\ | \qquad\quad O \\ HCOH \\ | \end{array}
&
\rightleftarrows
&
\begin{array}{l} HC{=}NH \\ | \\ HCOH \\ | \end{array}
\end{array}
$$

The hexosimines form pentaacetyl derivatives in which one acetyl group is connected to the nitrogen atom; the O-acetyl groups of the glucose derivative may be removed with the formation of N-acetylglucosylamine (acetamido N-glucoside).[2] This procedure is probably the best method for preparing the acetamide derivatives of the sugars, for direct combination has not yielded crystalline products. Since the N-acetylglucosylamine consumes two moles of periodic acid, it has a pyranose structure.[3]

[1] C. A. Lobry de Bruyn and A. P. N. Franchimont, *Rec. trav. chim.*, *12*, 286 (1893); E. J. Lorand, U. S. Patent 2,235,938, Mar. 25, 1941; I. E. Muskat, *J. Am. Chem. Soc.*, *56*, 693 (1934).

[2] P. Brigl and H. Keppler, *Z. physiol. Chem.*, *180*, 38 (1929).

[3] C. Niemann and J. T. Hays, *J. Am. Chem. Soc.*, *62*, 2960 (1940).

An isomeric acetamide derivative has been prepared by the action of ammonia on *aldehydo*-glucose pentaacetate;[4,5] lead tetraacetate oxidation shows that it has a furanose structure. Evidently, the ammonia combines with the aldehyde group and an acetyl group migrates to the amino group from the 4-position.

Diacetamide derivatives may result[4] from the action of ammonia on the acetylated nitriles (Wohl degradation), a process in which a carbon atom is lost. These substances probably have open-chain structures.

$$
\begin{array}{ccc}
\text{CN} & & \\
| & & \\
\text{HCOAc} & \xrightarrow{\text{NH}_3} & \text{HC(NHAc)}_2 \;+\; \text{NH}_4\text{CN} \\
| & & | \\
\text{AcOCH} & & \text{AcOCH} \\
| & & |
\end{array}
$$
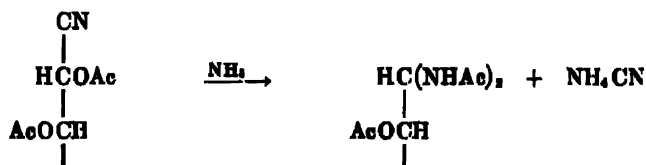
Although the ring forms exist, many of the properties of the glycosyl-amines are better interpreted on the basis of the imine structure, and it seems probable that the two structures are in equilibrium. In contrast to the desoxy sugar amines, in which the amino group replaces the primary or secondary hydroxyls, the osimines are hydrolyzed by dilute acids and are reduced to 1-amino alcohols.[6]

B. N-Glycosides. Schiff, in studying the reactions of amines with aldehydes, found that condensation products, the so-called Schiff bases, are formed.

$$\text{R}-\text{CHO} + \text{R}'-\text{NH}_2 \rightarrow \text{R}-\text{CH}=\text{N}-\text{R}' \quad (\text{Schiff base})$$

When the reaction first was applied to the sugars, amorphous products were obtained which were considered to have the Schiff base structure. Crystalline materials later were prepared[7] by heating glucose or fructose in an alcoholic solution of aniline. Sorokin believed these substances, frequently called sugar anilides, to have ring structures and to be analogous to the glycosides. It seems probable that the crystalline materials are N-glycosides but that in solution they are in equilibrium with the corresponding Schiff base and possibly with the anomeric (alpha-beta) and ring isomers.

$$
\begin{array}{ccccccc}
\text{RNHCH} & & & \text{RN}=\text{CH} & & & \text{HCNHR} \\
| & & & | & & & | \\
\text{HCOH} & \text{O} & \rightleftarrows & \text{HCOH} & \rightleftarrows & \text{HCOH} & \text{O} \\
| & | & & | & & | & |
\end{array}
$$
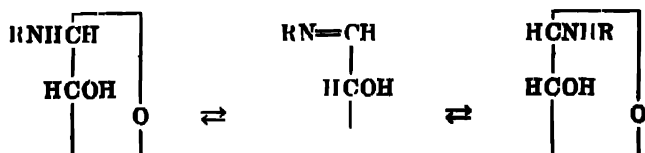
[4] R. C. Hockett and L. B. Chandler, *J. Am. Chem. Soc.*, **66**, 957 (1944).

[5] C. Niemann and J. T. Hays, *J. Am. Chem. Soc.*, **67**, 1302 (1945).

[6] A. R. Ling and D. R. Nanji, *J. Chem. Soc.*, **121**, 1682 (1922); W. Wayne and H. Adkins, *J. Am. Chem. Soc.*, **62**, 3314 (1940).

[7] B. Sorokin, *J. prakt. Chem.*, [2] **37**, 291 (1888).

In certain of their reactions, the substances behave as Schiff bases. However, since methylation and subsequent hydrolysis of aniline N-glucoside (glucose anilide) lead to tetramethylglucopyranose,[8] the compound probably has a pyranose ring.[9]

Certain o-nitroaniline derivatives of L-arabinose and D-ribose (I) have been shown to have ring structures.[10] They form triacetates, and all of the acetyl groups are removed by treatment with alcoholic ammonia, which does not hydrolyze N-acetyl groups. Because the ribose and arabinose derivatives form monotrityl derivatives, it is possible that the ring structures are furanoid, since it is known that trityl chloride reacts most easily with primary alcoholic groups. Two reaction products of ribose with aniline have

$$(HO)H_2C—CH—(CHOH)_2—CH—NH \quad (I)$$
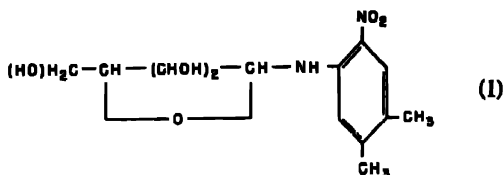
been isolated, both of which form triacetates.[11] A furanoid structure for one is indicated by the formation of a mono trityl derivative, the other may have a pyranoid structure, for it does not form a trityl derivative.

Although the evidence is not conclusive, it appears that the maltosyl-alkylamines may have an α-hydroxyamine structure similar to that of aldehyde-ammonias.[12]

$$R—CHO + R'—NH_2 \rightarrow R—CHOH—NH—R'$$
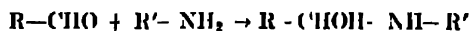
Because of the lack of knowledge of the structures of most of these compounds and of the isomerizations that take place in solution, the ring and open-chain structures will be used interchangeably in the present discussion, but the names will be based on the ring structures. The compounds have been called N-glycosides because the cyclic structures are analogous to those of the ordinary or O-glycosides. For the N-glycosides, the glycosidic linkage is through a nitrogen atom instead of an oxygen atom. In most cases it may be useful to follow the suggestion of Votoček and Valentin[14] and name them as substituted amines, e.g., methylamine N-glucoside = glucosyl-methylamine.

The glycosylamines have been of considerable interest because the nucleosides, hydrolytic products of the nucleic acids, are members of the

[8] Called 2,3,5,6-tetramethylglucose at the time.
[9] J. C. Irvine and R. Gilmour, J. Chem. Soc., 93, 1429 (1908).
[10] R. Kuhn and R. Strobele, Ber., 70, 773 (1937).
[11] L. Berger and J. Lee, J. Org. Chem., 11, 75 (1946)
[12] J. H. Werntz, U. S. Patent 2,181,929, Dec. 5, 1939
[14] E. Votoček and F. Valentin, Collection Czechoslov. Chem. Commun., 6, 77 (1934).

group. The discovery that several biologically important coenzymes are glycosylamines or their derivatives has greatly stimulated research in the field.

Glycosylamines from long-chain aliphatic amines such as dodecyl- and octadecylamine have been suggested as wetting agents and as textile softeners.[12] Those made by condensing D-glucose with aromatic amines are said to be useful antioxidants for rubber.[14] Since many important pharmaceuticals contain amino groups, it is possible to condense them with sugars and thus modify their biochemical action and solubility characteristics.

A number of glycosylamines have been tested as inhibitors of the growth of tubercle bacilli.[15] Because of the ease of dissociation of most glycosylamines into their components, it might be expected that the action would be similar to that of the free amine, except that the effective concentration might be greater. In general, the activity of glycosylamines parallels that of the free bases

a. PREPARATION

N-Glycosides having aliphatic amines and substituted anilines as aglycons are prepared simply by reaction of the amine and sugar, or acetylated sugar, in aqueous or alcoholic solution.[7,13 16] The addition of a small amount of acid may improve the yield but is likely to facilitate isomerization.

$$\text{Glucose} \xrightarrow[\substack{\text{H}_2\text{O} \text{ alcohol} \\ 20^\circ\text{C}}]{\text{aniline}} (\text{HO})\text{H}_2\text{C}\overline{\text{CH}(\text{CHOH})_3 \underset{\text{O}}{}\overset{\text{H}}{\underset{}{\text{C}}}\text{NH}\text{C}_6\text{H}_5}$$

$$\text{Pentaacetylglucose} \xrightarrow[\substack{\text{HOAc} \\ 20^\circ\text{C}}]{\text{toluidine}} (\text{AcO})\text{H}_2\text{C}\overline{\text{CH}(\text{CHOAc})_4 \underset{\text{O}}{}\overset{\text{H}}{\underset{}{\text{C}}}\text{NH}\text{C}_6\text{H}_4\text{CH}_3}$$
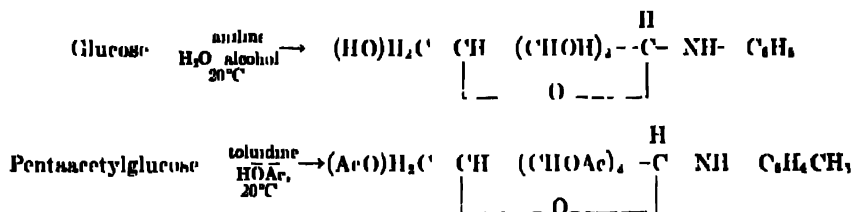
Depending upon the amine and the sugar involved, best yields are obtained by the selection of either ammonium chloride or hydrogen chloride as catalyst; in some instances, the addition of a catalyst is unnecessary and even undesirable.[17] Weakly basic amines, such as p-nitroaniline, will not react with the acetylated sugars although they form N-glycosides with the free sugars.[18]

[14] G. Kallner, U. S. Patent 1,862,270, June 7, 1932, W. S. Calcott and W. A. Douglass, U. S. Patent 1,796,980, Mar 17, 1931

[15] H. Lehr, H. Bloch and H. Erlenmeyer, *Helv. Chim. Acta*, **28**, 1415 (1945)

[16] M. Frèrejacque, *Compt rend*, **202**, 1190 (1936); K. Hanaoka, *J. Biochem (Japan)*, **31**, 95 (1940); F. Weygand, *Ber.*, **73**, 1259 (1940).

[17] R. Kuhn and L. Birkofer, *Ber.*, **71**, 621 (1938)

[18] M. Frèrejacque, *Compt rend*, **207**, 638 (1938)

From D-ribose, N-pyranosides are formed at room temperature whereas N-furanosides (the stable isomers) are produced when the solutions are refluxed.[19]

Of particular interest are the N-glycosides with two amino groups in the aglycon. Such substances are intermediates in the synthesis of isoalloxazine derivatives similar to riboflavin (vitamin B_2), the hydrogen-transporting coenzyme. The o-nitroaniline N-glycosides, prepared by the reaction of o-nitroaniline and sugars, are reduced by hydrogen in the presence of alkyl amines to the o-phenylenediamine N-glycosides.[20] An alternative procedure involved coupling substituted-aniline N-glycosides with diazonium salts and reducing the resulting azo dyes with hydrogen and nickel, or zinc and acetic acid, to substituted o-phenylenediamine N-glycosides.[21] The 1,2-diamino-4,5-dimethylbenzene N-glycosides react with alloxan to form flavin glycosides (see Riboflavin synthesis, p. 395)

o-Phenylenediamine reacts with two moles of glucose to form the di-glucosyl derivative or with one mole to give a derivative in which both amino groups are substituted.[22] Under oxidizing conditions, a benzimidazole
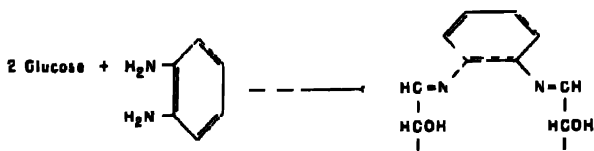
structure is produced. Griess and Harrow report that at least four compounds are formed from glucose and o-phenylenediamine. The structures of
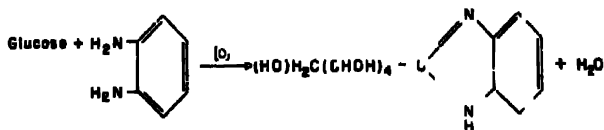
these compounds need study particularly in light of the present knowledge of the Amadori rearrangement (p 386).

A better method for the preparation of these derivatives involves the reaction of the aldonic and saccharic acids with o-phenylenediamine:
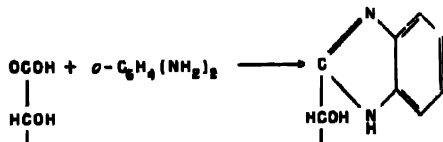
[19] L Berger and J Lee, J Org. Chem , 11, 84 (1946); J. Lee, U V Solmssen and L Berger, U S Patent 2,384,102, Sept 4, 1945
[20] Brit. Patent 461,215, Feb 8, 1937
[21] P. Karrer, U. S. Patent 2,237,074, Apr 1, 1941
[22] P Griess and G Harrow, Ber , 20, 281, 2205, 3111 (1887); B. Schilling, ibid., 34, 902 (1901).

Xylobenzimidazole forms the 2,5-anhydro derivative when heated with zinc chloride.[23] The benzimidazoles are useful for the characterization of the sugars and of the aldonic, saccharic and uronic acids.[24]

From glucosone, compounds with quinoxaline structures are produced.[25]

(a quinoxaline)
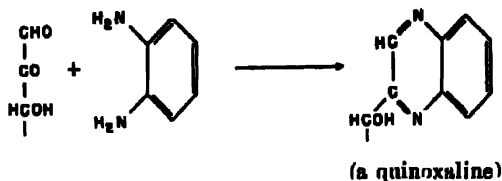
Urea, thiourea and guanidine condense directly with glucose under conditions similar to those used for the amines.[26]

$$\text{Glucose} + H_2NCONH_2 \rightarrow HC\!=\!N\!-\!CO\!-\!NH_2$$
$$|$$
$$HCOH$$
$$|$$

Glucose urea
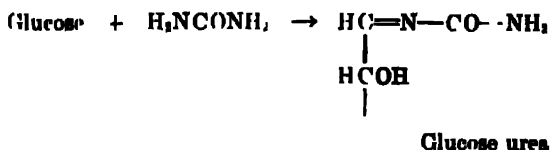
The urea derivative forms a pentaacetate upon acetylation with acetic anhydride and zinc chloride. Since one acetyl group is bound to a nitrogen atom, the compound probably has a ring structure; otherwise, a hexaacetate would be expected

The urea N-glucoside reduces Fehling solution much more slowly than D-glucose. The Barfoed reagent is not affected in thirty seconds at 100°C. Upon treatment with phenylhydrazine, the compound is converted to the osazone but more slowly than for glucose

The salts and lactones of the aldonic and saccharic acids react readily with phenylhydrazine to form the hydrazides.[27] The low solubility and ease of crystallization of the hydrazides has led to their use for the characterization and isolation of the acids. Aniline reacts in a manner similar to phenylhydrazine.

$$\text{OCOH} \quad + \text{ RHN}-NH_2 \quad \rightarrow \text{OC}-NH-NHR$$
$$|$$
$$HCOH$$
$$|$$
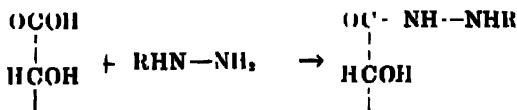
[23] C. F. Huebner, R. Lohmar, R. J. Dimler, S. Moore and K. P. Link, *J. Biol Chem.*, *159*, 503 (1945).

[24] S. Moore and K. P. Link, *J. Biol Chem.*, *133*, 293 (1940).

[25] H. Ohle, *Ber.*, *67*, 155 (1934).

[26] N. Schoorl, *Rec. trav. chim.*, *22*, 31 (1903); R. S. Morrell and A. E. Bellars, *J Chem. Soc.*, *91*, 1010 (1907); B. Helferich and W. Kosche, *Ber.*, *59*, 69 (1926); K. Quehl, U. S. Patent 2,116,640, May 10, 1938.

[27] L. Maquenne, *Bull. soc chim.*, [3] *43*, 719 (1887); E. Fischer and F. Passmore, *Ber.*, *22*, 2728 (1889)

Many aminopyrimidines do not condense directly with sugars. The lack of reactivity may be due to tautomerism of the amidine type:
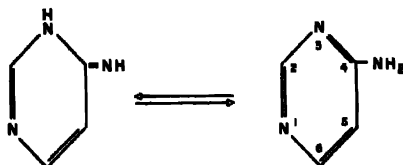
(4-Aminopyrimidine)

However, 4,6-diamino-2-methylpyrimidine in alcoholic solution reacts with xylose to give 6-amino-4-D-xylosylamino-2-methylpyrimidine.[28]

N-Glucosides formed from sulfanilamide are of interest because of the pharmacological importance of the aglycon.[29] They may be prepared by the reaction of the aglycon with glucose and are split, *in vivo*, with the liberation of sulfanilamide. A novel type of compound derived from sulfanilamide is prepared by the reaction of glucosamine and N-acetylsulfanilyl chloride. The resulting 2-desoxy-2-sulfanilylamino-D-glucose (I) has no effect on experimental streptococcal or pneumococcal infection in mice, and the bacteriostatic action is low. In 50 per cent acetic acid, the free amino group reacts with the reducing group of the glucosamine to form the corresponding N-glycoside (II).
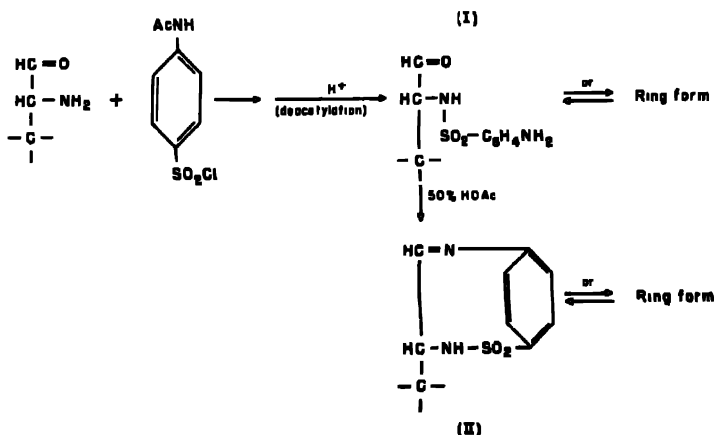
N-Glucosides, reported for sulfapyridine, sulfamethylthiazole and sulfaguanidine, contain two moles of sugar.[30] The biological action of the products is similar to that of the aglycons except that, for the sulfapyridine derivative, activity against cholera organisms was shown.

[28] J. Baddiley, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 571 (1943)

[29] Many references to the preparation and properties of these compounds are given by E. L. Jackson, *J. Am. Chem. Soc.*, 64, 1371 (1942).

[30] S. I. Lur'e and M. M. Shemyakin, *J. Gen. Chem.* (U.S.S.R.), 14, 935 (1944); *Chem. Abst.*, 39, 4597 (1945).

An important method of synthesis is based on the reaction of the acetyl-glycosyl halides with nitrogenous compounds or their metallic salts. De-acetylation gives the free N-glycoside.

$$\begin{array}{ccc} \text{HC}\overline{\text{Br}} & | & \\ | & \text{O} + \text{RNH}_2 \longrightarrow \\ \text{HOAc} & | & \end{array} \qquad \begin{array}{cc} \text{HC}\overline{\text{NHR}} & | \\ | & \text{O} \\ \text{HCOAc} & | \end{array}$$
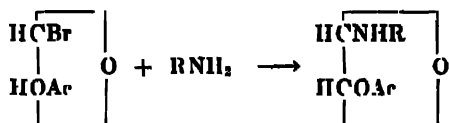
The silver salts of purines and pyrimidines react in this way to give N-glycosides.[31] These compounds are synthetic nucleosides. (Naturally occurring nucleosides are discussed later in this chapter.)

The acetylglycosyl halides react with silver cyanate or thiocyanate when refluxed in xylene solution to give derivatives with —NCO or —NCS groups in place of the halogen atom.[42] The products generally are amorphous but are valuable intermediates for the preparation of N-glycosides of the urea and hydantoin series, and possibly of the pyrimidine series, although attempts to prepare the latter compounds have been unsuccessful. The sugar isocyanates react with ammonia to produce urea N-glycosides and with alcohols to give urethans. The sugar isothiocyanates yield the corresponding thio derivatives

(Ac Gl) NCO + NH₃ → (Ac—Gl) NH—CO—NH₂   (Ac—Gl is the acetylated

(Ac Gl) —NCS + C₂H₅OH → (Ac Gl) NH CS OC₂H₅   glycosyl group)
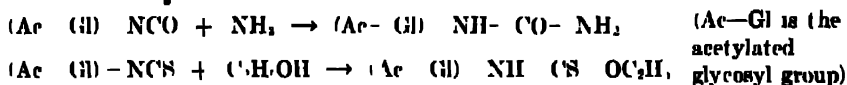
Tetraacetylglucosyl isothiocyanate (I) reacts with glycine ethyl ester hydrochloride to give tetraacetylglucosyl ethyl thiohydantoate (II) which on desulfuration and saponification is converted to glucosylhydantoin (III) or to glucosylhydantoic acid (IV).[33]

(Ac Gl)- N=C=S + H₂NCH₂COOR $\xrightarrow{\text{HCl}}$

(I)

(Ac Gl) NH— CS NH—CH₂COOR $\xrightarrow[\text{ROH}]{\text{AgNO}_3}$

(II)

(Ac Gl) NH CO NH- CH₂COOR $\xrightarrow{\text{KOH}}$ Gl NH—CO NH CH₂COOK

(IV) Gl NH CO NH CH COOH $\xleftarrow[\text{acid}]{\text{cold}}$ / hot acid

Gl- N CO

OC   (III)

HN - CH₂
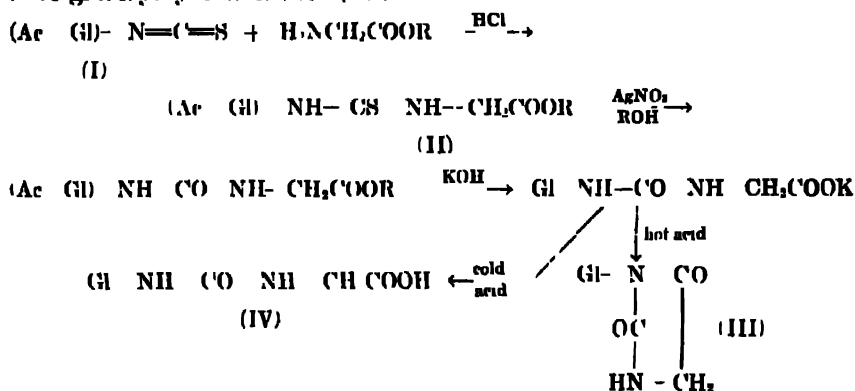
[31] E. Fischer and B Helferich, Ber, 47, 210 (1914), P. A Levene and J Compton, J Biol Chem, 114, 9 (1936), 117, 37 (1937).

[42] E. Fischer, Ber, 47, 1377 (1914); T B. Johnson and W Bergmann, J. Am. Chem. Soc., 60, 1916 (1938).

[43] K Haring and T B Johnson, J Am Chem Soc, 55, 395 (1933)

By condensation of acetylglycosyl bromides with *potassium* thiocyanate (instead of *silver* thiocyanate), the glycosyl thiocyanates are produced (instead of the isothiocyanates).

$$(Ac-Gl)-Br + KCNS \rightarrow (Ac-Gl)-SCN$$

At higher temperatures, rearrangement of the thiocyanate may take place with the formation of the isothiocyanate [34]

$$(Ac-Gl)-SCN \xrightarrow{140°C} (Ac-Gl)--NCS$$
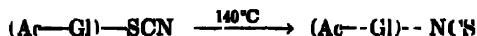
Potassium thiocyanate and strong hydrochloric acid react with aldose sugars to give compounds which appear to have a μ-thiolglucoxazoline structure[35] (III or II). The products are oxidized by H_2O_2 to the corresponding μ-hydroxyglucoxazolines.

(III)   (II)
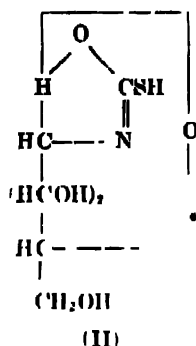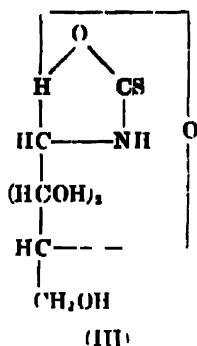
Nitrogenous bases may react directly with acetylglycosyl bromides to form N-glycosides. In this manner 1-glucosylcytosine (I) has been prepared.[36] With more basic nitrogenous substances the reaction is likely to

(I)
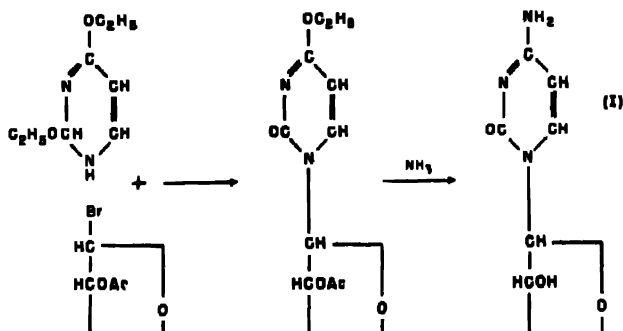
lead to the production of 1,2-glycoseens (see under Glycoseens). The action of diethylamine on tetraacetylglucosyl bromide leads to tetraacetyl-

[34] A. Muller and A Wilhelms, *Ber.*, **74**, 698 (1941)

[35] G. Zemplén, A. Gerecs and M. Rados, *Ber*, **69**, 718 (1936); W H Bromund and R. M. Herbst, *J. Org Chem*, **10**, 267 (1945)

[36] G E. Hilbert and E. F Jansen, *J Am. Chem. Soc*, **58**, 60 (1936)

1,2-glucoseen[37] or, depending on the conditions, to diethylamine tetraacetyl-N-glucoside.[38]

Nicotinamide (3-pyridine carboxamide) condenses with tetraacetylglucosyl bromide to give nicotinamide tetraacetyl-N-glucoside hydrobromide which is readily reduced in the aromatic nucleus by $Na_2S_2O_4$ (sodium dithionite) to 1,2 or 1,6-dihydro derivatives.[39] The reduced and deacetylated N-glucoside has absorption bands identical with those of dihydrocozymase, the hydrogen-transporting coenzyme of many biological systems (p. 393), and it is oxidized by the flavin coenzyme in the presence of air. The corresponding pyridine N-glucosides have absorption curves different from those of the cozymase.

b. REACTIONS OF N-GLYCOSIDES

The reactions of the N-glycosides are dependent to a considerable extent on the nature and basicity of the nitrogenous base involved. Unfortunately, the reactions have usually not been considered from this standpoint; hence, it is difficult to make generalizations.

All N-glycosides are hydrolyzed by acids. The pyrimidine N-glycosides, however, are so resistant to hydrolysis that the sugar may be destroyed in the process; this stability is decreased by hydrogenation of the pyrimidine nucleus. Very dilute acids bring about an isomerization of the N-glycosides of primary aromatic amines to ketose derivatives (Amadori rearrangement). Acetylated N-glycosides are readily hydrolyzed by dilute acetic acid, and only the nitrogenous base is removed; this procedure provides a method for the preparation of partially acetylated sugars in which the reducing group is free.[40] The natural purine and pyrimidine N-glycosides are fairly stable in the presence of alkali and do not reduce Fehling solution, but many synthetic N-glycosides exhibit a considerable reducing action.

Many N-glycosides exhibit mutarotation which may be due to the establishment of an equilibrium between the alpha and beta isomers and the corresponding Schiff base or possibly to a partial hydrolysis.[41]

$$
\begin{array}{ccc}
\begin{array}{c} \text{RHNCH} \\ | \\ \text{HCOH} \end{array}\Big| \,O\, \Big| & \rightleftharpoons \quad \begin{array}{c} \overset{N\ R}{\underset{}{\overset{\|}{C}}} - H \\ | \\ \text{HCOH} \end{array} \quad \leftarrow & \begin{array}{c} \text{HCNHR} \\ | \\ \text{HCOH} \end{array}\Big| \,O
\end{array}
$$

Schiff base
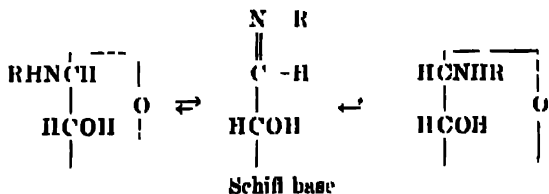
[37] K. Maurer, Ber , 62, 332 (1929)

[38] J. W. Baker, J. Chem. Soc , 1205 (1929)

[39] P. Karrer, B. H Ringier. J Büchi, H. Fritssche and U. Solmssen, Helv Chim Acta, 20, 55 (1937).

[40] J Lee and L. Berger, U S Patent 2,384,104, Sept 4, 1945.

[41] J. C. Irvine and R Gilmour, J. Chem. Soc., 93, 1429 (1908); 95, 1545 (1909); R. Kuhn and L. Birkofer, Ber , 71, 1535 (1938); J W. Baker, J. Chem. Soc., 1205 (1929)

The mechanism outlined necessitates the presence of a hydrogen atom attached to the nitrogen atom, i.e., the aglycon amine must be a primary amine. However, the observed mutarotation of the corresponding derivatives of secondary amines may be ascribed to the formation of an intermediate quaternary ion: $R_2N^+{=}CH-(CHOH)_4-$ CH_2OH.

The postulation of an equilibrium between the N-glycosides and the corresponding Schiff bases is substantiated by the addition of HCN to form nitriles:[42]

$$R-N{=}CH-(CHOH)_4-CH_2OH + HCN \rightarrow R \overset{\displaystyle CN}{\underset{\displaystyle |}{NH}} CH(CHOH)_4-CH_2OH$$

N-Furanosides mutarotate to equilibrium values different from those for the corresponding N-pyranosides.[43] However, if heated in alcoholic solution, the pyranosides are converted to furanosides.

c. AMADORI REARRANGEMENT

Amadori[44] reported that the product initially formed from D-glucose and p-toluidine was very labile and isomerized in the presence of acids into a "stable" form. The "labile" isomer was thought to be the N-glucoside and the "stable" isomer the Schiff base. However, the "stable" isomer gives positive color reactions for ketoses; it is reduced to N-p-tolylmannamine (III) and it forms a hydroxylamine derivative.[45] From this evidence, it is clear that an isomerization from a D-glucose (I) to a D-fructose (II) derivative has taken place. This is called the Amadori rearrangement.


```
HC=N -C₆H₄ - CH₃          H₂C -NH—C₆H₄  CH₃          H₂C NH -C₆H₄ - CH₃
 |                   H⁺    |                    H₂      |
HCOH            →         CO             →         HOCH
 |                         |                         |

  I        (Amadori        II                       III
       rearrangement)
```
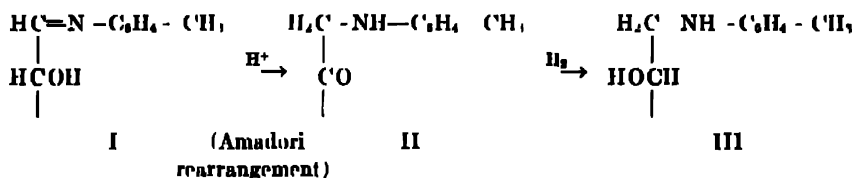
The reaction is catalyzed by hydrogen ions. It seems to be general for N-glycosides of primary aromatic amines, but it fails to occur for the corresponding derivatives of alkylamines.[46] The catalytic effect of hydrogen ions on the conversion makes it probable that the reaction takes place through the cation of the Schiff base (II p. 387) and the sugar enol (III) which rearranges to give the 1-substituted ketose (IV).[46a]

---

[42] W. v. Miller and J Plochl, Ber., **27**, 1281 (1894); E Votoček and O Wichterle, Coll. Czechoslov. Chem Commun , **9**, 109 (1937)

[43] L Berger and J Lee, J Org Chem , **11**, 75 (1946).

[44] M. Amadori, Atti accad. Lincei, [6] **2**, 337 (1925); **13**, 72, 195 (1931), C N. Cameron, J. Am. Chem. Soc., **48**, 2737 (1926).

[45] R. Kuhn and F. Weygand, Ber., **70**, 769 (1937)

[46] a. F. Weygand, Ber , **73**, 1259 (1940); b E Mitts and R M. Hixon, J. Am. Chem Soc., **66**, 483 (1944)

RNH₂⁺ ... structures (I) (II) (III) (IV)
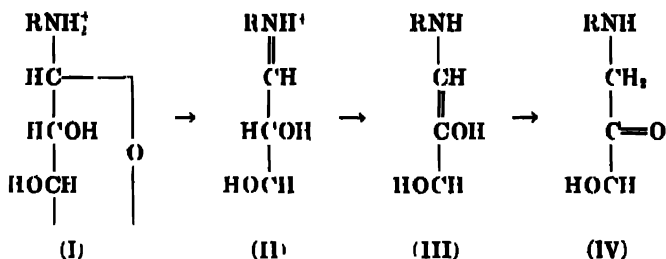
Hydrogenation of the ketose derivative (IV) produces 1-desoxy-1-(arylamino) sugar alcohols. Since a new asymmetric center is produced, two isomeric alcohols may be formed, but the yield of the two possible isomers is influenced greatly by the acidity of the medium employed for the hydrogenation.[47] In acid solution catalytic reduction of 1-desoxy-1-p-toluidinefructose (V) takes place only in the aromatic ring (VI); but in alkaline or neutral solution, it takes place with the formation of 1-desoxy-1-p-toluidinemannitol (p-tolyl-D-mannamine) (VII).

(VII) (V) (VI)
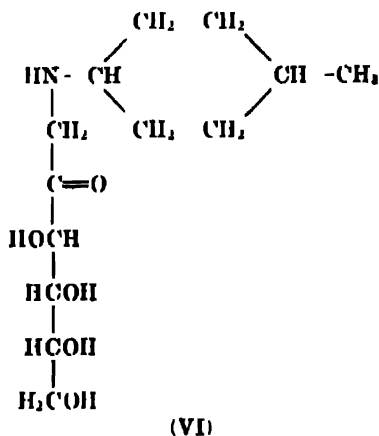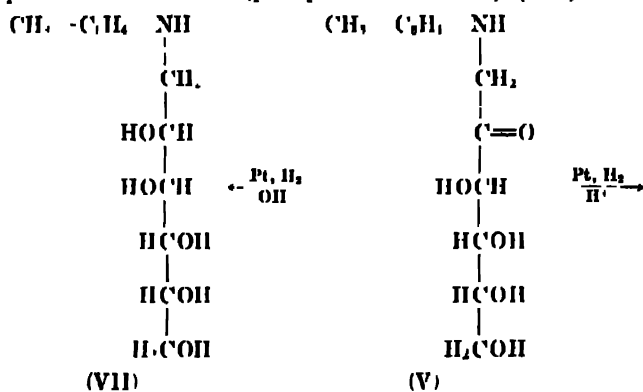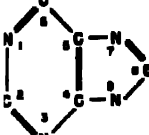
[47] F. Weygand, Ber., 73, 1259, 1278 (1940).

However, for 1-desoxy-1-*p*-toluidine-L-ribulose, acid reduction yields 1-desoxy-1-toluidine-L-arabitol whereas alkaline reduction produces 1-desoxy-1-toluidine-L-ribitol.[48] These reactions provide a new method for the production, from the readily available N-arabinosides, of 1-(N-substituted)-ribitol derivatives of the type of riboflavin. The reactions are also of interest in providing a possible mechanism for the *in vivo* formation of riboflavin.

d. Nucleosides[49]

Partial hydrolysis of the widely distributed nucleic acids produces a group of N-glycosides called nucleosides. These are combinations of purines or pyrimidines with D-ribose or 2-desoxy-D-ribose. The structures of the purines and pyrimidines most commonly found in nucleosides (and in the nucleic acids) are outlined below and the names of the corresponding nucleosides are given.

Aglycon type	Naturally occurring aglycons	Corresponding Nucleoside	Chemical Structure of Aglycon
	Cytosine	Cytidine	1-Amino-2-pyrimidone
	Uracil	Uridine	2,6-Pyrimidinedione
	Thymine	Thymidine	5-Methyl-2,6-pyrimidinedione (5-methyluracil)

Pyrimidine nucleus

	Adenine	Adenosine	6-Aminopurine
	Guanine	Guanosine	2-Amino-6-purinone
	Hypoxanthine	Inosine	6-Purinone

Purine nucleus

Several other compounds of the nucleoside type have been isolated from vetch seeds.[50] Vicine, believed to be 2,5-diamino-4,6-pyrimidinedione N-glucoside, is accompanied by a similar nucleoside, convicine. From yeast extracts, an adenine N-thiomethylpentoside, probably the 5-thiomethylriboside, has been isolated.[51]

Preparation of Nucleosides. Levene and Jacobs[52] treated yeast nucleic acid with ammonia in an autoclave (175°C.) and isolated several crystalline

[48] F. Weygand, *Ber.*, *73*, 1259 (1940).

[49] General references: See under Nucleic Acids.

[50] H. Ritthausen, *J. prakt. Chem.*, [2] *24*, 202 (1881); *29*, 359 (1884); H. J. Fisher and T. B. Johnson, *J. Am. Chem. Soc.*, *54*, 2038 (1932).

[51] U. Suzuki and T. Mori, *Biochem. Z*, *162*, 413 (1925); G. Wendt, *Z. physiol Chem.*, *272*, 152 (1942)

[52] P. A. Levene and W. A. Jacobs, *Ber.*, *43*, 3154 (1910). See also P. A. Levene and L. W. Bass, under Nucleic Acids.

ribonucleosides: adenosine (adenine N-ribofuranoside), guanosine (guanine N-ribofuranoside), cytidine (3-cytosine N-ribofuranoside) and uridine (3-uracil N-ribofuranoside). The method has been improved by Phelps[63] by the use of magnesium oxide rather than ammonia. Almond emulsin contains enzymes which hydrolyze nucleic acids to the nucleosides, and it has been used for their preparation.[64] Picric acid, which forms double salts particularly with adenosine, is useful for separating the products of hydrolysis. The picric acid is removed as the potassium salt.[65]

The enzymic synthesis of inosine and guanosine from the corresponding purines and ribose 1-phosphate has been accomplished using enzymes (nucleoside phosphorylases) obtained from the liver tissue of rats.[66] The synthesis is of particular interest because it probably represents the process by which nucleosides are synthesized naturally.

Structure of Nucleosides. All of the known nucleosides from nucleic acids have furanose structures. This type of ring was shown for guanosine and adenosine by methylation and subsequent hydrolysis to a trimethylribose. The latter substance is oxidized by nitric acid to inactive dimethyl-*meso*-tartaric acid[67] and, hence, is 2,3.5-trimethylribose. Bredereck[68] showed that uridine, cytidine, adenosine and inosine react with trityl chloride. Since the reagent reacts preferably with primary alcoholic groups, the substances were assigned furanose structures. Methyl ribopyranoside does not react with trityl chloride under the conditions employed; hence, this conclusion seems to be justified although it is known that secondary alcoholic groups will react under drastic conditions. The trityl group can be replaced with a tosyl group (after substitution of the free hydroxyls by methyl or acetyl groups) and finally with iodine.[69] Since the replacement of a tosyl group with iodine (by reaction with sodium iodide in acetone) goes readily only with esters of primary alcohols, the furanose structure receives additional support.

There has been some doubt concerning the position of attachment of the purines to the sugars of purine N-ribosides and N-desoxyribosides. Levene's arguments for the 7-position in the purine nucleus apply equally well to the 9-position (see p. 388 for structure), but he preferred the former. Inasmuch as comparison of the absorption spectra[60] of the nucleosides

[63] F. P. Phelps, U. S. Patent 2,152,662, Apr 4, 1939.

[64] H. Bredereck, A Martini and F. Richter, *Ber.*, 74, 694 (1941).

[65] H. Bredereck, *Ber*, 71, 1013 (1938).

[66] H. M. Kalckar, *Federation Proc.*, 4, 248 (1945).

[67] P. A. Levene and R S. Tipson, *J. Biol. Chem.*, 94, 809 (1932); 97, 491 (1932).

[68] H. Bredereck, *Z. physiol. Chem.*, 225, 61 (1934)

[69] P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, 105, 419 (1934); 109, 623 (1935); 121, 131 (1937).

[60] J. M. Gulland and E. R. Holiday, *J. Chem. Soc.*, 765 (1936); J. M. Gulland and L. F. Story, *ibid.*, 692 (1938).

with those of the corresponding 7- and 9-methyl-aglycons shows correspondence of the 9-methyl purines with the nucleosides the sugars are probably attached through the nitrogen atom at position 9 of the aglycon. This structure is also confirmed by evidence from methylation studies.[61] The ring connection of uridine, a pyrimidine derivative, was shown[61,62] by methylation and hydrolysis to be at position 3, for the product of the hydrolysis is 1-methyluracil, and the only other nitrogen atom is at position 3.

O=C—CH=CH   O=C CH=CH   (Gl' = 5 trityl
 | (1)   (8) | | | 2,3-diacetyl-
HN—C-- N Gl' ──CH₃N₂→ CH₃ N C   N Gl'   ribofuranosyl
   ‖   ‖   group)
   O   O
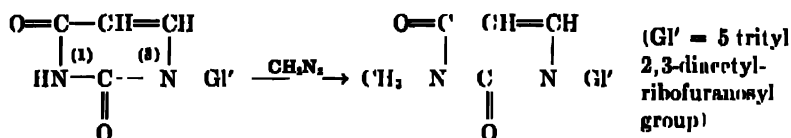
The nucleosides containing desoxyribose are prepared from thymus nucleic acids by enzymic cleavage. One successful method requires passing thymus nucleic acid through a segment of the gastrointestinal tract of a dog and collecting the product from an intestinal fistula.[63] Dry emulsins from the mucosa of the small intestine and liver are more suitable for general application.[64]

The sugar present in the thymus nucleosides is 2-desoxy-D-ribose, identical with the synthetic substance prepared from D-arabinose and arabinal.[65] The principal evidence for the type of ring structure of these nucleosides is that trityl derivatives are formed.[66] Hence, the sugar rings are probably of the furanose type.

2. Nucleotides[67]

A. Preparation and Structure. Careful partial hydrolysis of nucleic acids produces nucleotides (phosphorylated nucleosides) which are composed of one mole each of sugar, phosphoric acid and a purine or pyrimidine base. The hydrolytic conditions must be mild; otherwise, the nucleosides will be produced through the loss of phosphoric acid. Alkaline or enzymic hydrolysis of yeast nucleic acid gives two purine and two pyrimidine nucleotides; acid, however, degrades the two purine compounds.[68] The pyrimidine

[61] H. Bredereck, G. Muller and E Berger, *Ber.*, **73**, 1058 (1040).

[62] P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **104**, 385 (1934).

[63] P. A. Levene and E. S. London, *J. Biol. Chem.*, **83**, 793 (1929)

[64] S. J. Thannhauser and M. Angermann, *Z. physiol. Chem.*, **186**, 13 (1929); F. Bielschowsky and W. Klein, *ibid.*, **207**, 202 (1932).

[65] P. A. Levene, L. A. Mikeska and T. Mori, *J. Biol. Chem.*, **85**, 785 (1929–30).

[66] P. A. Levene and R. S Tipson, *J Biol. Chem*, **109**, 623 (1935).

[67] See. J. M. Gulland, *J. Chem. Soc*, 208 (1944).

[68] H. S. Loring and F. H Carpenter, *J. Biol. Chem.*, **150**, 381 (1943); H. Steudel. *Z. physiol. Chem.*, **188**, 203 (1930); P. A. Levene, *J. Biol. Chem.*, **40**, 415 (1919).

nucleotides are so much more stable than the purine derivatives that the two types may be separated by the action of acids. The brucine salts have value for the separation of the four nucleotides from yeast nucleic acid. However, the great difference in solubility in pyridine may be used as the basis for the separation of the pyrimidine nucleotides.[69] Adenylic acid is best isolated as the aluminum picrate salt.[70]

The general constitution of the purine nucleotides is demonstrated by their hydrolysis by acids to a purine and ribose (or 2-desoxyribose) monophosphate and by alkalies to the nucleosides and phosphoric acid. Hence, the order of the constituents in a purine nucleotide must be:

$$
\begin{array}{cc}
\text{H}^+ & \text{OH}^- \\
\downarrow & \downarrow
\end{array}
$$

purine- sugar- phosphoric acid
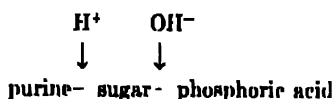
Because of their acid nature, the nucleotides are often named as acids, e.g., adenylic acid (nucleoside is adenosine), inosinic acid (nucleoside is inosine), etc.

Muscle adenylic acid and inosinic acid, found free in yeast or tissue extracts, have the phosphoric acid residue at carbon 5 of the sugar whereas the nucleotides obtained by the degradation of nucleic acids are esterified at carbon 3. The structure of the ribose mono phosphate obtained by acid hydrolysis of inosinic acid is shown by its oxidation by nitric acid to phosphoribonic acid.[71] Since nitric acid oxidizes primary alcoholic as well as free reducing groups, phospho-ribo-trihydroxyglutaric acid (and not phosphoribonic acid) would be produced if the phosphoric acid group did not block the primary hydroxyl on carbon 5. This evidence is confirmed by the synthesis of inosinic acid by the phosphorylation (with $POCl_3$) of hypoxanthine 2,3-monoisopropylidene-N-ribofuranoside followed by the removal of the isopropylidene group.[72] Inasmuch as muscle adenylic acid may be deaminated with the production of inosinic acid,[73] both compounds must be esterified in the same positions.

The adenylic acid obtained by the hydrolysis of yeast nucleic acid differs from the adenylic acid of muscle tissue extracts. The deaminated acid hydrolyzes spontaneously in aqueous solution, and a ribose monophosphate may be isolated. This product cannot be substituted at positions 1, 4 or 5 because it is oxidized to a phosphoribonic acid and forms both furanosides and

[69] H. Bredereck and G. Richter, Ber., 71, 718 (1938)

[70] M. V. Buell, J. Biol. Chem., 150, 389 (1943).

[71] P. A. Levene and W. A. Jacobs, Ber., 44, 746 (1911)

[72] P. A. Levene and R. S. Tipson, J. Biol. Chem., 111, 313 (1935)

[73] G. Embden and G. Schmidt. Z. physiol. Chem., 181, 130 (1929); G. Schmidt, ibid., 179, 243 (1928).

pyrauosides. An ingenious method was devised to distinguish between positions 2 and 3. Thus, the phosphoribose was reduced by hydrogen and platinic oxide to a phosphoribitol. Since this reduced product is optically inactive, it must be the 3-phosphoribitol (a meso compound). The isomer substituted at position 2 would be active. Hence, yeast adenylic acid is 3-phosphoadenosine.[71] The synthesis[75] of yeast adenylic acid from adenosine by methods which phosphorylate preferentially the hydroxyl of carbon 3 adds some support for the 3-phosphoadenosine structure. The pyrimidine nucleotides from yeast nucleic acid are assumed to have similar structures since they form trityl derivatives and since their solutions in the presence of boric acid do not have a greater conductivity than boric acid alone[76] (no contiguous hydroxy groups; see p. 49). Uridylic acid has been synthesized by the reaction of trityluridine with diphenylphosphoryl chloride and subsequent removal of the trityl and phenyl groups.[77]

B. Adenosine Di- and Tri-phosphoric Acids. Extracts of muscle tissue contain a compound which is adenosine esterified with three moles of phosphoric acid.[78] Acid hydrolysis of the adenosine triphosphoric acid produces one mole of adenosine, one of ribose monophosphate and two moles of phosphoric acid. Neutral hydrolysis, however, gives muscle adenylic acid and pyrophosphoric acid.[79] Lohmann has suggested the formula illustrated for the adenosine triphosphoric acid, but definite proof has not been given and other formulas have been proposed.[80] Since the structures of other biologically important substances depend on this formula, it is important that additional evidence be obtained.

Support for the Lohmann formula for adenosine triphosphoric acid is given by studies[81] of adenosine diphosphoric acid (see below). The latter compound is hydrolyzed by an enzyme of snake venom (Russell's viper) with the liberation of pyrophosphate ($H_4P_2O_7$) and orthophosphate (H_3PO_4). The production of pyrophosphate would be expected from the Lohmann formula if the hydrolysis takes place at the point of esterification with the ribose residue.

[71] P. A. Levene and S. A. Harris, *J. Biol. Chem.*, **98**, 9 (1932); **101**, 419 (1933)

[75] G R. Barker and J. M. Gulland, *J. Chem. Soc.*, 231 (1942)

[76] H. Bredereck, *Z. physiol. Chem.*, **224**, 79 (1934)

[77] H. Bredereck and E. Berger, *Ber.*, **73**, 1121 (1940); J M Gulland and G J. Hobday, *J. Chem. Soc.*, 746 (1940).

[78] C H Fiske and Y Subbarow, *Science*, **70**, 381 (1929), K Lohmann, *Naturwissenschaften*, **17**, 624 (1929)

[79] K. Lohmann, *Biochem. Z.*, **282**, 109, 120 (1935).

[80] H K. Barrenscheen and W. Filz, *Biochem. Z.*, **250**, 281 (1932); T. Satoh, *J Biochem* , **21**, 19 (1935).

[81] J M Gulland and E Walsh, *J. Chem. Soc.*, 169 (1945).
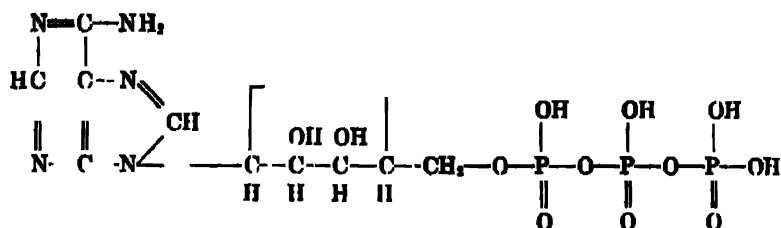
Adenosine triphosphoric acid

(Lohmann)

By the action of enzymes, one phosphoric acid group is removed from adenosine triphosphoric acid, and adenosine diphosphoric acid is formed. These two acids are extremely important because they form part of the phosphorylating system of yeast fermentation and of the anaerobic conversion of glycogen to lactic acid in animals. In the presence of certain proteins from yeast or muscle and of magnesium ion, transesterifications with sugars or degradation products take place and the adenosine triphosphoric acid may give up a phosphoric acid group or one may be taken up by the adenosine diphosphoric acid.

R—OH + Adenosine triphosphoric acid $\rightleftarrows$ R—O—P=O

+ Adenosine diphosphoric acid
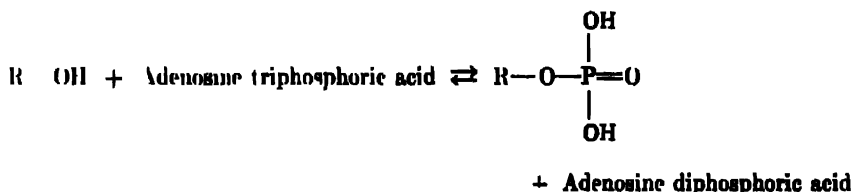
These acids are coenzymes for many biological reactions in which phosphorylation takes place.

C. Biologically Important Substances Related to the Nucleotides. As defined, the nucleotides are purine or pyrimidine N-glycosides esterified with phosphoric acid (N-base-sugar-phosphoric acid). Several vitamins of the B-group and coenzymes have closely similar structures with different aglycons, with ribitol instead of ribose, or with other differences.

Cozymase. There occurs in yeast and in muscle tissue a dialyzable substance (coenzyme) which is necessary for the *in vitro* fermentation of sugars by yeast extracts. Concurrent work[82] in the laboratories of Warburg and Euler established the following formula for the cozymase (CoZ) (also called coenzyme I, codehydrogenase I or diphosphopyridine nucleotide)

[82] O. Warburg, W. Christian and A. Griese, *Biochem. Z.*, **282**, 157 (1935); H. v. Euler and F. Schlenk, *Z. physiol. Chem.*, **246**, 64 (1937).

Nicotinic amide   D-Ribose   Adenylic acid

Cozymase or Coenzyme I
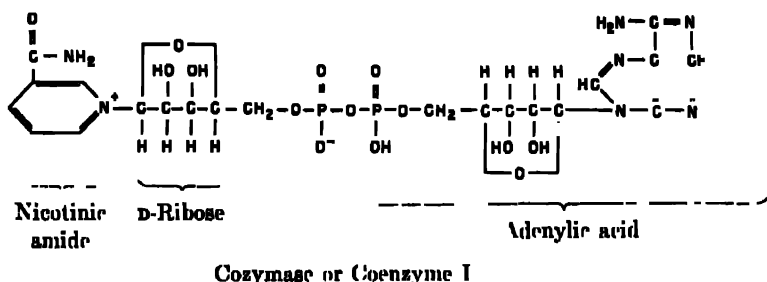
The cozymase might be considered as a mixed dinucleotide consisting of adenylic acid and a second nucleotide-like compound which has one of the B-complex vitamins (nicotinic amide) as the aglycon. Almond emulsin hydrolyzes cozymase, and nicotinamide N-riboside may be separated from the hydrolyzate.[83]

The cozymase functions as a hydrogen acceptor, or donor in the reduced form, and as the coenzyme for many biological oxidation-reduction reactions. Two hydrogen atoms are consumed per mole and dihydrocozymase is produced. The hydrogen atoms are probably taken up by saturation of the double bond of the quaternary nitrogen of the nicotinic amide ring. The reaction takes place only in the presence of specific proteins which form easily dissociable compounds with the cozymase. Although the cozymase acts as a hydrogen acceptor for many biological reactions, the associated protein varies with the reaction involved. Some 35 different enzymic reactions are known for which cozymase acts as the coenzyme. Negelein and Wulff[84] crystallized a protein which acts with cozymase (CoZ) to dehydrogenate ethyl alcohol or reduce acetaldehyde. The reaction may be represented:

$$C_2H_5OH + CoZ \xrightleftharpoons{\text{protein}} CH_3CHO + CoZ H_2$$
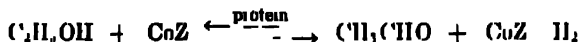
Other alcohols are also oxidized by this enzyme system. Although cozymase is also required for the conversion of 1,3-diphosphoglyceraldehyde to 1,3-diphosphoglyceric acid, the corresponding protein (apoenzyme) is different from that necessary for the reduction of acetaldehyde.[85]

In reactions of this type there is some confusion as to what may be called an enzyme. Neuberg and Euler[86] express the equilibrium between the coenzyme and protein as:

Apoenzyme + Coenzyme ⇄ Holoenzyme

[83] F Schlenk, *Arch. Biochem.*, **3**, 93 (1943).
[84] E Negelein and H. J. Wulff, *Biochem. Z.*, **293**, 351 (1937).
[85] O Warburg and W. Christian, *Biochem Z*, **301**, 221 (1939); E. Negelein and H. Bromel, *ibid.*, **301**, 135 (1939).
[86] C. Neuberg and H. v. Euler, *Biochem. Z.*, **240**, 245 (1931).

Some investigators consider the protein-coenzyme complex (holoenzyme) as the enzyme whereas others reserve the term for the protein (apoenzyme).[87]

Coenzyme II. This substance (codehydrogenase II or triphosphopyridine nucleotide) occurs in many tissue and plant extracts and fulfills the same function as the cozymase (coenzyme I) of acting as an acceptor of hydrogen or as a donor of hydrogen when in the reduced form. It seems to have a structure similar to that for the coenzyme I but the molecule contains a third molecule of phosphoric acid, the position of which is disputed. Enzymes exist which phosphorylate the coenzyme I to coenzyme II.[61][88]

Riboflavin. Another coenzyme for oxidation-reduction reactions is the phosphorylated riboflavin. Riboflavin, also known as vitamin B₂, or lactoflavin, has been synthesized.[89] The structure and a method of synthesis are illustrated.

(I) (II) (III)

Alloxan   Riboflavin (Lactoflavin, vitamin B₂;
           6,7-dimethyl 9 D-ribityl isoalloxazine)
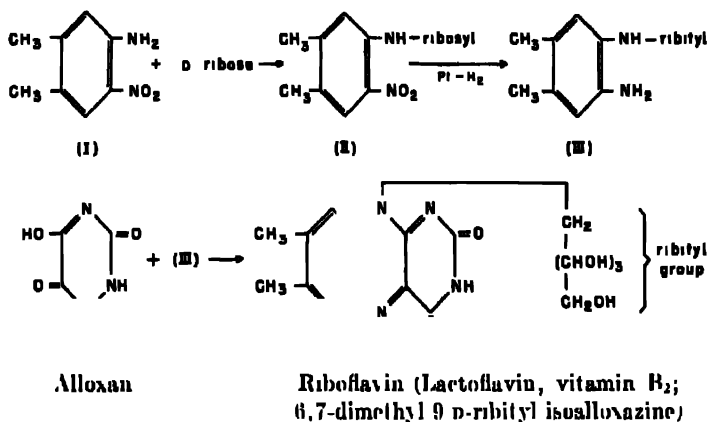
Of particular interest is the occurrence of D-ribitol in the vitamin. Riboflavin was identified by Warburg and Christian as a constituent of the "yellow enzyme" first crystallized by Theorell. The yellow enzyme, another of the oxidation-reduction enzymes, acts apparently as an intermediate between coenzyme I or II and oxygen. It takes up hydrogen from the reduced forms of the coenzyme I or II and gives it up to oxygen directly or through the intermediary of hematin compounds (the cytochromes). Theorell succeeded in separating the "yellow enzyme" into a protein and a

[87] See: M. Dixon, *Ann. Rev. Biochem.*, *8*, 20 (1939); J. O. Parnas, *Am. Rev. Soviet Med.*, *1*, 485 (1944).

[88] H. v. Euler and E. Adler, *Z. physiol. Chem.*, *252*, 41 (1938).

[89] P. Karrer, K. Schöpp and P. Benz, *Helv. Chim. Acta*, *18*, 426 (1935); R. Kuhn, K. Reinemund and R. Ströbele, *Ber.*, *68*, 1765 (1935); F. Bergel, A. Cohen and J. W. Haworth, *J. Chem. Soc.*, 165 (1945); M. Tishler, J. W. Wellman and K. Ladenburg, *J. Am. Chem. Soc.*, *67*, 2165 (1945).

phosphorylated riboflavin and in recombining them. Neither of the components, which are in equilibrium in solution, has any action when alone. The natural phosphorylated riboflavin was shown by the following series of reactions to have the phosphate group at carbon 5 of the ribitol residue.[90]

R
|
CH₂
|
(CHOH)₃
|
CH₂OH

— TrCl →

R
|
CH₂
|
(CHOH)₃
|
CH₂OTr

$\xrightarrow{(Ac)_2O \atop NaOAc}$

R
|
CH₂
|
(CHOAc)₃
|
CH₂OTr

— — →

R
|
CH₂
|
(CHOAc)₃
|
CH₂OH

$\xrightarrow{POCl_3}$,

R
|
CH₂
|
(CHOAc)₃
|
CH₂OPO₃H₂

(R = substituted isoalloxazine radical)
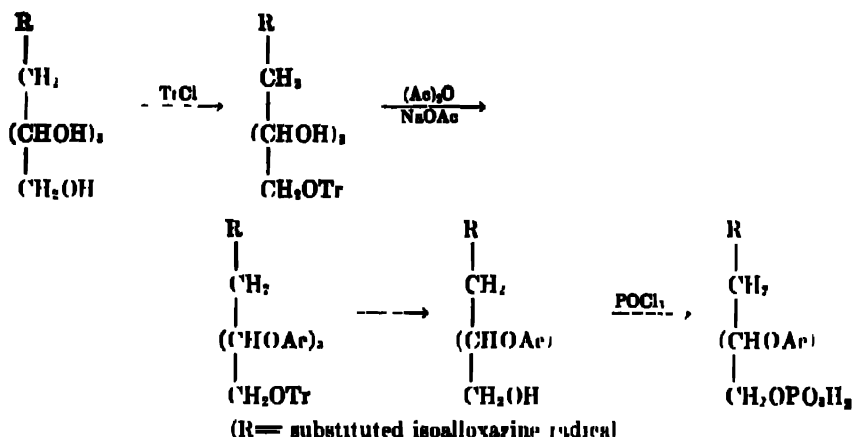
The final product was shown to be identical with the triacetate obtained by acetylation of the natural material

Diaphorase. In the yeast fermentation system and other biological systems, still another compound called diaphorase (or "coenzyme factor") may act as the intermediary for the transfer of hydrogen from reduced cozymase to the hematin compounds.[91] The substance is a dinucleotide of adenylic acid and riboflavin phosphate.

$$\text{Adenine—(D-ribose)} - \text{O}-\underset{\underset{O}{\|}}{\overset{\overset{OH}{|}}{P}}-\text{O}-\underset{\underset{O}{\|}}{\overset{\overset{OH}{|}}{P}}-\text{O - riboflavin} \quad \text{(Diaphorase)}$$
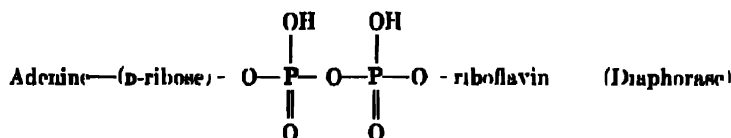
The diaphorase is another coenzyme; depending upon the specific protein present (apoenzyme), it may act as an amino acid oxidase, as a receptor of hydrogen from reduced cozymase or possibly as a xanthine oxidase

3. Nucleic Acids[67,92,94]

The nucleic acids or polynucleotides are of considerable biological importance since they are constituents of all cells. In combination with

[90] R Kuhn, H. Rudy and F. Weygand, *Ber.*, **69**, 1543 (1936).

[91] H v Euler and H. Hellstrom, *Z. physiol. Chem.*, **252**, 31 (1938); J. G. Dewan and D. E Green, *Biochem. J.*, **32**, 626 (1938).

[94] General references: T. B. Johnson, "Organic Chemistry," p. 1005; Editor H Gilman John Wiley and Sons, New York (1938). P A Levene and L. W. Bass,

proteins, they make up the nucleoproteins. The crystalline viruses have been identified as nucleoproteins. The nucleotide or nucleoside nature of several B-complex vitamins and coenzymes makes it likely that a biological relationship exists between these various substances.

Sugars, phosphoric acid and nitrogenous bases (purines and pyrimidines) are the ultimate hydrolysis products of the nucleic acids. Peculiarly enough, the sugar components are the rare D-ribose and 2-desoxy-D-ribose. For a long time, the nucleic acids were used as the source of D-ribose (see under D-ribose in Chapter III). With the possible exception of L-lyxose which has been reported[51] to be present in the hydrolysis products of yeast nucleic acid, no other sugars are known to occur in nucleic acids. The nitrogenous bases which are found in the hydrolysis products are the pyrimidines: cytosine, 5-methylcytosine, uracil and thymine; and the purines: adenine and guanine (see p. 388 for the formulas of these bases).

Partial hydrolysis by alkali or by enzymes produces nucleotides and finally nucleosides. These partial hydrolysis products are described in preceding sections. The nucleotides are composed of one mole each of phosphoric acid, sugar and purine or pyrimidine, and the nucleosides of one mole of the sugar and purine or pyrimidine.

Nucleic acid
↓
Nucleotides
↓
Nucleosides + H₃PO₄
↓
Purines or pyrimidines + D-ribose or 2-desoxyribose
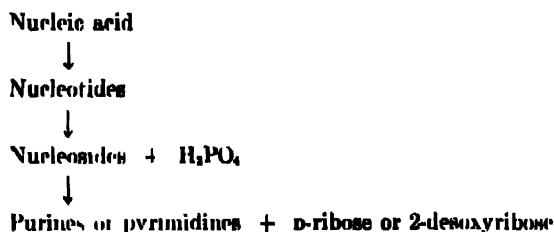
The two chief types of nucleic acids are represented by the yeast and thymus nucleic acids. The two types have been distinguished as "plant" and "animal" nucleic acids according to their supposed occurrence. It now seems more probable that both types occur in all living cells. Although it has been suggested that nucleic acids of the thymus type are nuclear constituents and those of the yeast type are cytoplasmic constituents,[54] the morphological studies of Caspersson and Schultz[55] indicate that the acids of the yeast type originate in the nucleus and diffuse into the cytoplasm.

"Nucleic Acids;" A.C.S. Monograph No. 56, Chemical Catalog Co., New York (1931)
R. S. Tipson, *Advances in Carbohydrate Chem.*, 1, 193 (1945).

[51] For methods of preparation, see: L. Laufer, U. S. Patent 2,379,912, July 10, 1945; S. Redfern, U. S. Patent 2,387,040, Oct. 16, 1945.

[54] J. M. Gulland and G. R. Barker, *J. Chem. Soc.*, 625 (1943).

[55] M. Behrens, *Z. physiol. Chem.*, 253, 185 (1938).

[56] T. Caspersson and J. Schultz, *Proc. Natl. Acad. Sci. U. S.*, 26, 507 (1940).

Since the principal known difference between the several nucleic acids lies in the nature of the carbohydrate component, they may be divided into ribonucleic acids (formerly plant nucleic acids) and desoxyribonucleic acids (animal nucleic acids).[97] As indicated by the name, the former yield ribose and the latter 2-desoxyribose on hydrolysis. These substances are not well defined and their homogeneity has often been questioned. Although generally considered as tetranucleotides, the naturally occurring products may be of high molecular weight[98] and the products actually isolated vary greatly in their degree of polymerization.[99]

A. Thymus Desoxyribonucleic Acid. Enzymic hydrolysis of thymus nucleic acid gives small amounts of four desoxyribose nucleosides: guanine, adenine, cytosine and thymine N-2-desoxyribosides.[100] Acid hydrolysis of the nucleic acid leads sometimes to the isolation of mono and diphosphoric acid esters of 2-desoxyribosyl thymine and cytidine. These results are interpreted as indicating that the thymus nucleic acid consists of four nucleosides (two pyrimidine and two purine) connected by means of phosphoric acid linkages.[101]

Thymus desoxyribonucleic acid
(according to Levene)
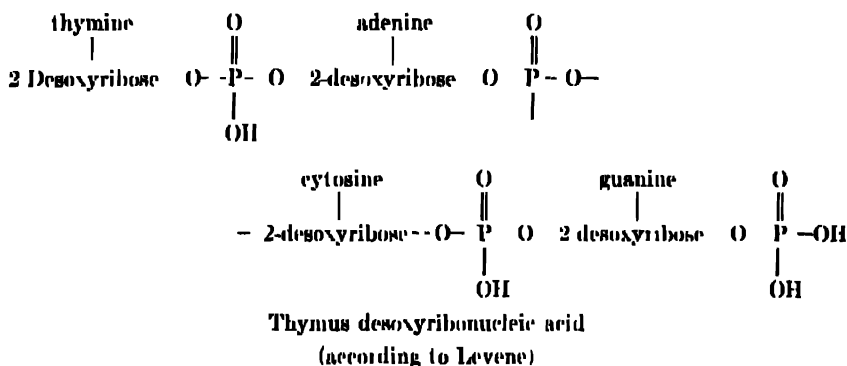
The Levene formula agrees with the results of electrometric titrations[102] and with the production of diphosphoric acid esters of pyrimidine N-2-desoxyribosides. Makino[104] proposes a cyclic formula similar to the Levene

[97] F. W Allen, *Ann. Rev. Biochem*, *10*, 221 (1941). See additional suggestions by A. W. Pollister and A. E. Mirsky, *Nature*, *152*, 692 (1943).

[98] R. Signer, T Caspersson and E. Hammarsten, *Nature*, *141*, 122 (1938)

[99] G Schmidt, E. G. Pickels and P. A. Levene, *J. Biol. Chem.*, *127*, 251 (1939); W. E. Fletcher. J. M. Gulland, D O Jordan and H. E. Dibben, *J Chem. Soc.*, 30 (1944).

[100] P. A. Levene, *J. Biol. Chem.*, *48*, 119 (1921); H. Bredereck and G. Caro, *Z. physiol. Chem.*, *253*, 170 (1938).

[101] P A Levene and R. S. Tipson, *J. Biol. Chem.*, *109*, 623 (1935)

[102] P. A. Levene and H S. Simms, *J. Biol. Chem.*, *70*, 327 (1926).

[104] K. Makino, *Z. physiol Chem*, *296*, 201 (1935).

structure, while Thannhauser[104] suggests that the linkage between the nucleotide units is formed by condensation between phosphoric acid units.

Thymus desoxyribonucleic acid
(according to Thannhauser)
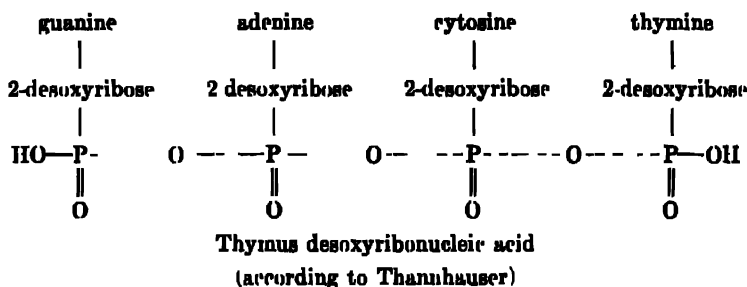
B. Yeast Ribonucleic Acid. The yeast nucleic acid yields on alkaline hydrolysis four nucleosides: guanine, adenine, cytosine and uracil N-D-ribosides, or, under milder alkaline conditions, the four corresponding nucleotides. Electrometric titrations carried out by Levene and Simms[105] indicated the presence of four primary and one secondary phosphoric acid groups. With these results as the principal sustaining evidence, Levene proposed the following formula which is analogous to that for the thymus nucleic acid.

Yeast ribonucleic acid
(according to Levene)
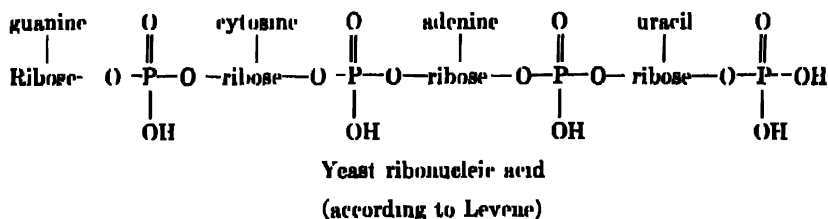
Probably because of the amorphous nature of the nucleic acid and of the consequent difficulty of purification, later workers report different titration data. Fletcher, Gulland and Jordan[106] find their preparations to have four ionizable hydrogen atoms, one of which provides a secondary dissociation. Since they have demonstrated that their product is a polymerized tetranucleotide, their titration data could be interpreted as indicating that the tetranucleotide as formulated by Levene polymerizes by condensation between a phosphate group of one tetranucleotide and a hydroxyl group (sugar hydroxyl at carbon 2?) of a second tetranucleotide. However, from other data, these workers also find it necessary to modify the Levene

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[104] See: W. Klein and A. Rossi, Z. physiol. Chem., **231**, 104 (1935).

[105] P. A. Levene and H. S. Simms, J. Biol. Chem., **70**, 327 (1926).

[106] W. E. Fletcher, J. M. Gulland and D. O. Jordan, J. Chem. Soc., **33** (1944); J M Gulland and E Walsh, ibid , 172 (1945).

formula by connecting two of the individual nucleotide units to the phosphate group of a third nucleotide unit.

Takahashi[107] reported that enzymes which hydrolyze monoesters of phosphoric acid have no effect on nucleic acid although, according to the Levene formula, it has one singly esterified phosphoric acid group. (This observation is disputed by Klein who was unable to reproduce the earlier results of Takahashi.) Makino[108] measured the titration curve for the nucleic acid and found evidence for four primary hydrogens and *no* secondary hydrogens. To fit this evidence, Takahashi and Makino propose a cyclic formula.
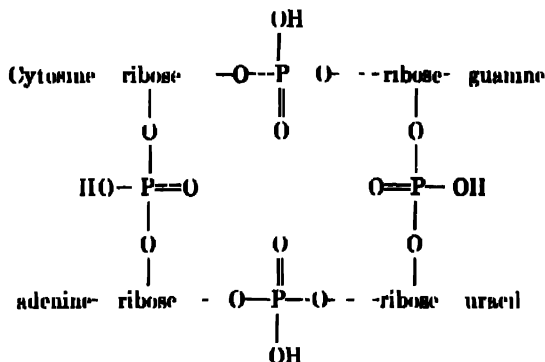
Yeast ribonucleic acid

(according to Takahashi and Makino)

The linkage between the nucleotide units cannot involve amino groups of purine and pyrimidine bases, for deamination of the yeast and thymus nucleic acids takes place without concurrent hydrolysis. As a result of a study of the products of partial hydrolysis of yeast nucleic acid by the action of aqueous pyridine, Bredereck and associates[109] have also provided information on the arrangement of the nucleotides in the nucleic acids. Thus, partial hydrolysis produces guanylic acid and a trinucleotide which liberates adenylic acid on additional hydrolysis. This information, which agrees best with the Levene type of formula, indicates that the guanylic acid is at one end of the tetranucleotide and that the cytidylic and uridylic acids are connected together. Bolomey and Allen[110] have obtained similar results by partial enzymic hydrolysis.

[107] H. Takahashi, *J. Biochem. (Japan)*, *16*, 463 (1932).

[108] K. Makino, *Z. physiol. Chem.*, *236*, 201 (1935); F. W Allen and J J. Euler, *J. Biol. Chem.*, *137*, 757 (1941).

[109] H. Bredereck, E. Berger and F. Richter, *Ber.*, *74*, 338 (1941); P. A Levene and W. A. Jacobs, *ibid.*, *43*, 3150 (1910).

[110] R. A Bolomey and F. W. Allen, *J. Biol. Chem.*, *144*, 113 (1942).

As is obvious from the previous considerations, the position of the phosphoric acid residues connecting the nucleotides is still in doubt. Levene and Tipson, because of the much greater stability of the thymus nucleic acid to alkaline hydrolysis as compared with the yeast nucleic acid, consider that the hydroxyls of carbons 3 and 5 of neighboring desoxyribose units of the thymus nucleic acid are connected, for yeast nucleic acid, the hydroxyls of carbons 2 and 3 of the ribose units probably are involved.

C. Nucleic Acid of Tobacco Mosaic Virus. The crystalline virus is a nucleoprotein which on hydrolysis yields 5 to 6 per cent of a ribonucleic acid.[111] The freshly isolated nucleic acid has an average particle weight of about 300,000 and decomposes spontaneously to particles with a molecular weight of about 61,000.[112] Both products are highly asymmetric

4. Reactions of the Sugars With Substituted Hydrazines and Hydroxylamine

Hydrazines (R—NH—NH$_2$), hydroxylamine (NH$_2$OH), semicarbazide (H$_2$N—NHCONH$_2$), and other nitrogenous bases react with sugars in a manner somewhat similar to that of the amines. Many of the products mutarotate in solution and exist as ring forms and as acyclic derivatives analogous to the Schiff base isomers of the N-glycosides. The most important of these sugar derivatives are those prepared from phenylhydrazine and other hydrazines. The oximes are intermediates in the Wohl method of shortening the carbon chains of sugars, and both the oximes and semicarbazones have been utilized for the preparation of the acyclic aldehydo-sugars (p. 153)

A. Hydrazones and Osazones.[113] The reaction of phenylhydrazine with the sugars was discovered by Fischer[114] and was extensively employed in the classical work which established the configuration of the sugars. The products obtained have been widely employed for characterization and identification although they are somewhat difficult to purify, and the melting points are often decomposition points.[115]

Hydrazones. When one mole each of phenylhydrazine and sugar react, phenylhydrazones are formed. Most hydrazones are water-soluble, but the mannose phenylhydrazone is so insoluble that it may be used for the quan-

[111] F. S Bawden and N W. Pirie, *Proc. Roy. Soc. London, B* **123**, 274, (1937); H S. Loring, *J. Biol. Chem.,* **130**, 251 (1939).

[112] S S. Cohen and W M. Stanley, *J. Biol Chem.,* **144**, 589 (1942).

[113] A. W. van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren," Gebrüder Borntraeger, Berlin (1920).

[114] E. Fischer, *Ber.,* **17**, 579 (1884).

[115] E Fischer, *Ber.,* **41**, 73 (1908).

titative estimation of mannose. The hydrazones are often of value for the separation of sugars, for they may be converted to the original sugars by treatment with benzaldehyde or with concentrated hydrochloric acid.

$$
\begin{array}{ccccc}
\text{HC=O} && \text{HC=N—NH—Ph} && \text{HC=O} \\
| & \xrightarrow{\text{H}_2\text{N—NH—Ph}} & | & \xrightarrow{\text{Ph—CHO}} & | \quad + \\
\text{HCOH} && \text{HCOH} && \text{HCOH} \\
| && | && \\
&&&& \text{Ph—CH=N—NH—Ph}
\end{array}
$$
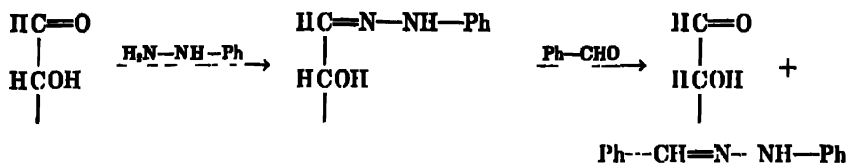
Substituted hydrazones less soluble than the phenylhydrazones are usually employed. A hydrazine which is reported[116] to show great specificity in reacting only with *aldoses* of certain configurations has the following formula: $H_2N—N(CH_3)—C_6H_4—CH_2—C_6H_4—N(CH_3)—NH_2$. Substituted hydrazines suitable for the identification of some important sugars are as follows:

p-Bromphenylhydrazine in acetic acid solution for mannose, fucose and arabinose and probably ribose and talose.

α-Methylphenylhydrazine for arabinose, fucose, mannose, galactose and talose.

p-Nitrophenylhydrazine for arabinose, rhamnose, fucose, glucose, fructose, mannose, galactose and glucuronic acid.

The conditions best adapted for identification purposes are described in detail by van der Haar.[113] The formation of the hydrazones takes place most rapidly at pH 4 to 5 and in the presence of high concentrations of buffer. Phosphate ion is reported to have a greater catalytic effect than acetate ion[117] and hydrochloric acid catalyzes hydrazone but not osazone formation, particularly in the absence of air.[118]

Ardagh and Rutherford[117] find the reaction to be of second order whereas Compton and Wolfrom[119] report it to be pseudo-monomolecular when a hydrazine hydrochloride solution buffered with acetate ions is used.

Information valuable for the interpretation of the structure of the hydrazones is provided[119] by the reaction of α-methylphenylhydrazine with tetraacetylgalactopyranose (I), tetraacetylgalactofuranose (II) and *aldehydo*-pentaacetylgalactose (III). As all the hydrazones formed are converted to the same pentaacetylgalactose methylphenylhydrazone (IV) when acetylated, it appears that the hydrazones have open-chain structures.

[116] J. v. Braun and O. Bayer, *Ber*, **58**, 2215 (1925); F. L. Humoller, S. J. Kuman and F. H Snyder, *J. Am. Chem. Soc.*, **61**, 3370 (1939).

[117] E. G. R. Ardagh and F. C. Rutherford, *J. Am. Chem. Soc.*, **57**, 1085 (1935).

[118] A. Orning and G. H. Stempel, Jr , *J. Org. Chem.*, **4**, 410 (1939); G. H. Stempel, Jr., *J. Am. Chem. Soc.*, **56**, 1351 (1934).

[119] J. Compton and M. L. Wolfrom, *J. Am Chem Soc.*, **56**, 1157 (1934)

(I)   (IV)

```
         (I)                                                              (IV)

     ┌─────────┐
     │ HOCH    │           HC=N—N(CH₃)—Ph              HC=N—N(CH₃)—Ph
     │   │     │               │                          │
     │ HCOAc   │           HCOAc                       HCOAc
     │   │     │               │                          │
     │AcOCH  O │    ──→     AcOCH             (Ac)₂O     AcOCH
     │   │     │               │            ─────────→     │
     │ AcOCH   │           AcOCH             pyridine    AcOCH
     │   │     │               │                          │
     │ HC──────┘           HCOH                        HCOAc
     │   │                     │                          │
     │ H₂COAc              H₂COAc               ──→    H₂COAc
```

(corresponding structures (I) → (IV) with acetylation via (Ac)₂O/pyridine)

```
     ┌─────┐
     │ HOCH│                HC=N- N(CH₃)- Ph │            HC=O
     │  │  │                    │            │              │
     │ HCOAc                HCOAc            │            HCOAc
     │  │  │                    │            │              │
  O AcOCH  │    ─→           AcOCH           │  (Ac)₂O    AcOCH
     │  │  │                    │            │ ─────────    │
     └──CH─┘                HOCH             │  pyridine  AcOCH
        │                      │             │              │
     HCOAc                  HCOAc            │            HCOAc
        │                      │                           │
     H₂COAc                 H₂COAc                       H₂COAc

      (II)                                                (III)
```
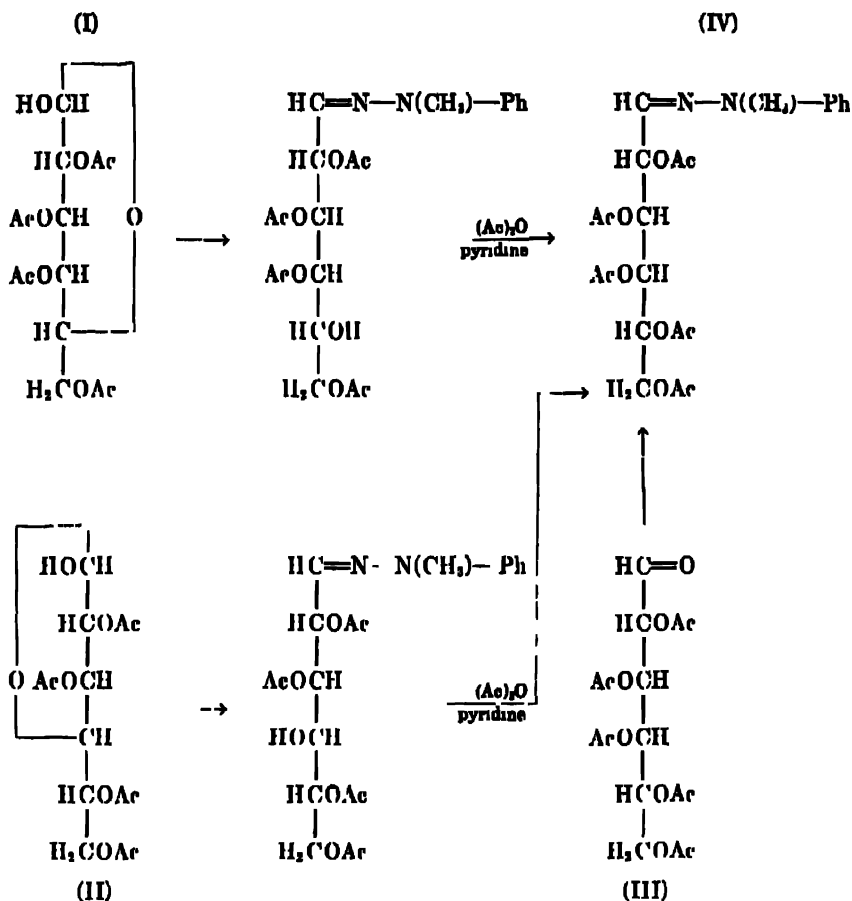
The rate of hydrazone formation for the three types of galactose acetates is very different:

	k
Aldehydo-galactose pentaacetate	0 054
Tetraacetylgalactofuranose	0 016
Tetraacetylgalactopyranose	0 00052

This difference in the rate of hydrazone formation makes it probable that the rate-determining reaction is either the opening of the rings (for the cyclic acetates) to form the acyclic derivatives or the direct reaction of the original substances with the substituted hydrazine.

Although the acetylated galactose hydrazones probably have acyclic structures, the hydrazones with free hydroxyls may exist in the ring forms.

In solution, the sugar hydrazones show complex mutarotations which pass through a maximum or minimum.[118,120,121] The failure of the mutarotation equation to follow the first-order equation indicates that three or more substances take part in the equilibrium. Two isomeric glucose phenylhydrazones exist,[122] and their structures have been extensively investigated by Behrend and collaborators. The so-called "beta" isomer is usually obtained, and the labile "alpha" isomer is easily transformed into the "beta" form. Behrend and Reinsberg[123] showed that the crystalline pentaacetate of the "α"-glucose phenylhydrazone has one acetyl group attached to a nitrogen atom because removal of the phenylhydrazine group gives acetylphenylhydrazine. Since one hydroxyl escapes acetylation, it is probably involved in ring formation, and the "α"-glucose phenylhydrazone is a cyclic isomer. The acetylated "beta" isomer, however, gives phenylhydrazine. This method of distinguishing between cyclic and acyclic acetyl derivatives has been improved by the development of methods for distinguishing between N-acetyl and O-acetyl groups (see below under Osazones).

Sugar Osazones. By treatment of sugars with an excess of phenylhydrazine at 100°C., two phenylhydrazine residues are introduced into the molecule, and sugar osazones, difficultly soluble in water, are formed.[124] Optimal conditions for the preparation of glucosazone have been determined.[125] The reaction proceeds most rapidly in the presence of acetate buffers at a pH of about 4 to 6; in more acid solution (particularly in the absence of air) and with the free base only the hydrazone is formed.[118,126] The presence of sodium bisulfite in the reaction mixture inhibits the formation of colored by-products.[127]

Osazone formation is favored by the presence of electron-attracting groups attached to the hydrazine radical and is inhibited by the presence of alkyl groups. Nitrophenylosazones are formed with ease under mild conditions. However, methylphenylhydrazine oxidizes only primary alcoholic groups under the usual conditions and forms osazones from ketoses much more easily than from aldoses.

The formation of osazones requires three moles of phenylhydrazine per mole of sugar but one mole is reduced during the reaction to yield one

[120] H. Jacobi, *Ann.*, **272**, 170 (1892)
[121] C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, **53**, 4358 (1931).
[122] Z. H. Skraup, *Monatsh.*, **10**, 401 (1889); C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, **51**, 3161 (1929).
[123] R. Behrend and W. Reinsberg, *Ann.*, **377**, 189 (1910)
[124] E. Fischer, *Ber.*, **17**, 579 (1884).
[125] D. D. Garard and H. C. Sherman, *J. Am. Chem. Soc.*, **40**, 955 (1918).
[126] J. Kenner and E. C. Knight, *Ber.*, **69**, 341 (1936).
[127] R. H. Hamilton, Jr., *J. Am. Chem. Soc.*, **56**, 487 (1934).

mole each of aniline and ammonia.[128] To explain the formation of the
aniline and ammonia, the following mechanism has been proposed:[129]

$$
\begin{array}{l}
\text{HC}=\text{O} \\
\quad | \\
\text{HCOH}
\end{array}
\xrightarrow{\text{Ph}-\text{NH}-\text{NH}_2}
\begin{array}{l}
\text{HC}=\text{N}-\text{NH}-\text{Ph} \\
\quad | \\
\text{HCOH}
\end{array}
\quad \underline{\text{Ph}-\text{NH}-\text{NH}_2},
$$

$$
\begin{array}{l}
\text{HC}=\text{N}-\text{NH}-\text{Ph} \\
\quad | \\
\text{C}=\text{O}
\end{array}
\quad \underline{\text{Ph}-\text{NH}-\text{NH}_2} \rightarrow
\begin{array}{ccc}
\text{HC}=\text{N} & \text{NH} & \text{Ph} \\
\\
\text{C}=\text{N} & \text{NH} & \text{Ph}
\end{array}
$$

$$
+ \text{NH}_3 + \text{C}_6\text{H}_5\ \text{NH}_2
$$
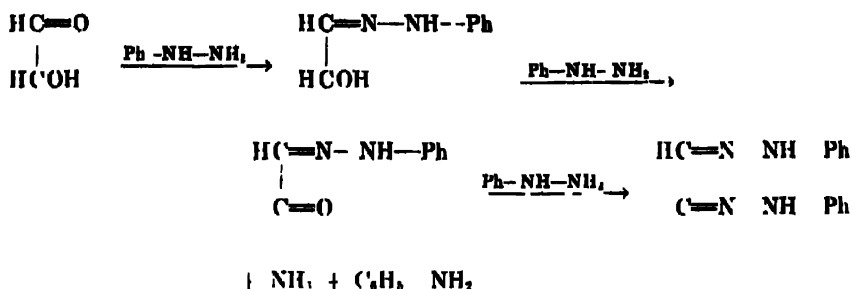
It seems unlikely that a reducing agent as mild as the secondary alco
holic group (at carbon 2) could reduce the phenylhydrazine, especially since
titanium trichloride does not Also, Weygand[130] has found that the 1-(N-
aryl)-fructoses react with phenylhydrazines, under the same conditions as
those which favor osazone formation, with the production of good yields
of the osazones The yields are often higher than in the usual procedure
starting with the sugar A new mechanism[130] involving the Amadori rear-
rangement, proposed to agree with the above observations, is illustrated
for the formation of a phenylosazone from an aldose hydrazone

$$
\begin{array}{l}
\text{Aldose} \\
\quad \downarrow \\
\text{H}_2\text{N}\ \text{NHPh}
\end{array}
\rightarrow
\begin{array}{l}
\text{HC}=\text{N}\ \text{NHPh} \\
\quad | \\
\text{HCOH}
\end{array}
\xrightarrow{\text{H}^+}
\begin{array}{l}
\text{HC}=\text{N}-\text{HNHPh} \\
\quad | \\
\text{HCOH} \\
\quad |
\end{array}
\rightarrow
$$

$$
\begin{array}{l}
\text{HC}\ \text{NH}\ \text{NHPh} \\
\quad \| \\
\text{COH}
\end{array}
\rightarrow
\begin{array}{l}
\text{HC}=\text{NH} \\
\quad | \\
\text{C}=\text{O}
\end{array}
\xrightarrow{2\text{H}_2\text{N}-\text{NHPh}}
\begin{array}{l}
\text{HC}=\text{N}\ \text{NHPh} \\
\\
\text{C}=\text{N}\ \text{NHPh}
\end{array}
$$

$$
+ \text{C}_6\text{H}_5\text{NH}_2 \qquad + \text{NH}_3 + \text{H}_2\text{O}
$$
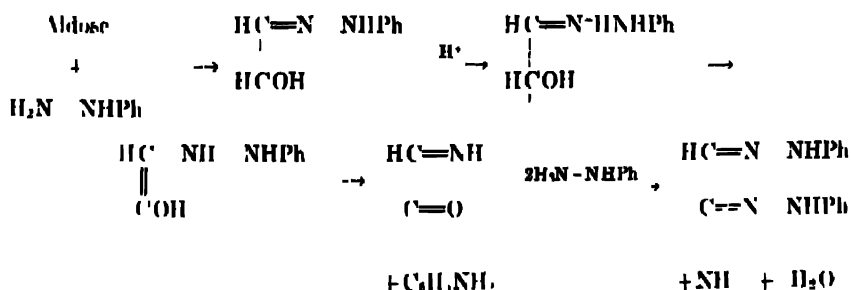
The phenylosazones of the sugars, because of their insolubility, are of
considerable value for the identification of the sugars. Since the asymmetry
of carbon 2 is destroyed in their preparation, the osazones of three related
sugars (the two epimers and the corresponding ketose) are identical

$$
\begin{array}{l}
\text{HCO} \\
\quad | \\
\text{HCOH}
\end{array}
\text{and}
\begin{array}{l}
\text{HCO} \\
\quad | \\
\text{HOCH}
\end{array}
\rightarrow
\begin{array}{l}
\text{HC}=\text{N}\ \text{NH}\ \text{Ph} \\
\quad | \\
\text{C}=\text{N}-\text{NH}-\text{Ph}
\end{array}
\leftarrow
\begin{array}{l}
\text{H}_2\text{COH} \\
\quad | \\
\text{C}=\text{O}
\end{array}
$$
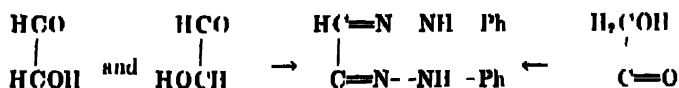
[128] E. Knecht and F. P. Thompson, *J Chem Soc*, *125*, 222 (1924).

[129] E. Fischer, *Ber*, *20*, 821 (1887).

[130] F Weygand, *Ber*, *73*, 1284 (1940)

There are but four D- and four (enantiomorphic) L-hexose phenylosazones and only two D- and two L-pentose derivatives. Thus, the preparation of the osazone of an unknown sugar may be utilized for the preliminary allocation of the unknown to a group of three possible sugars, and the final identification may be made on the basis of the preparation of difficultly soluble hydrazones which are characteristic of the individual sugars. (See above under Hydrazones.) Photomicrographs of many phenylosazones, of considerable value for identification purposes, are given by Hassid and McCready.[131] The rotations of the hydrazones and osazones are utilized for distinguishing between the D,L isomers, and for this purpose a mixture of two volumes of alcohol and three volumes of pyridine frequently has been used as a solvent.[132] Confirmation of the identity of the osazones is achieved by conversion to the corresponding osotriazoles (see below).

Although advantageous for the identification of the sugars, the phenyl-osazones are not applicable to the isolation of sugars. The phenylhydrazine groups are removed by treatment with benzaldehyde, concentrated hydrochloric acid or particularly well by pyruvic acid,[133] but the resulting product, a sugar osone, is a mixed ketose-aldose.

$$CH=N-NH-Ph \qquad\qquad HC=O$$
$$| \qquad\qquad\qquad\qquad\qquad\qquad\qquad |$$
$$C=N\ NH-Ph \qquad \xrightarrow{PhCHO} \qquad C=O$$
$$| \qquad\qquad\qquad\qquad\qquad\qquad\qquad |$$

Glucose phenylosazone   Glucosone
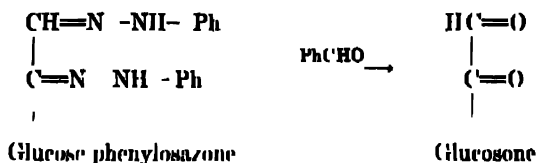
Since the sugar osazones mutarotate in alcoholic pyridine solution,[131] the classical formula for these substances may be questioned, and there is much evidence that they exist in cyclic as well as acyclic forms. The subject is complicated because migration of the double bond possibly takes place. The mutarotation has been ascribed to a partial hydrolysis of the osazones, and appreciable quantities of the sugar and hydrazine exist in the equilibrium solution.[135] This explanation is also supported by the ease with which the hydrazine radicals of the osazones are exchanged with hydrazine

---

[131] W. Z. Hassid and R. M. McCready, *Ind Eng Chem*, *Anal. Ed*, *14*, 683 (1942)

[132] It should be noted that in early work in this field it was often the custom to report the observed rotation rather than the calculated specific rotation. Also, the rotations given by Levene and LaForge, *J. Biol. Chem.*, *20*, 429 (1915), for a number of important osazones must be multiplied by 100 to give the correct values; cf F. W. Zerban and L. Sattler, *Ind. Eng. Chem.*, *34*, 1182 (1942).

[133] E. Fischer and E. F. Armstrong, *Ber.*, *35*, 3141 (1902); L. Brüll, *Ann chim. applicata*, *26*, 415 (1936).

[134] E. Zerner and R. Waltuch, *Monatsh.*, *35*, 1025 (1914).

[135] L. L. Engel, *J Am Chem Soc.*, *57*, 2419 (1935).

molecules in the solvent. When the second hydrazine is different from that used in making the osazone, mixed osazones are formed.[135,136]

$$
2 \quad
\begin{array}{l}
HC\!\!=\!\!N\!-\!NHR \\
\quad | \\
C\!\!=\!\!N\!-\!NHR \\
\quad |
\end{array}
+ 2\ NH_2\!-\!NHR' \rightarrow
$$

$$
\begin{array}{l}
HC\!\!=\!\!N\ \text{-}NHR' \\
\quad | \\
C\!\!=\!\!N\!-\!NHR \\
\quad |
\end{array}
+
\begin{array}{l}
HC\!\!=\!\!N\!-\!NHR \\
\quad | \\
C\!\!=\!\!N\!-\!NHR' \\
\quad |
\end{array}
+ 2\ RNH\!-\!NH_2
$$
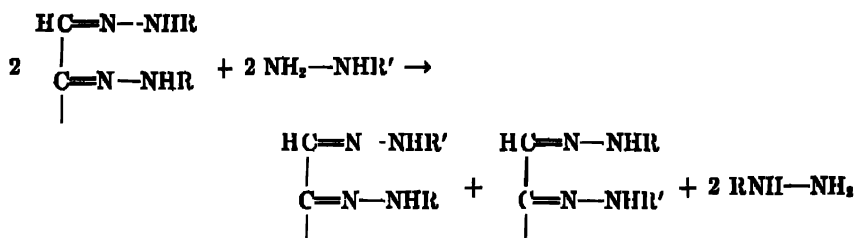
Mild acetylation of the glucose and galactose phenylosazones leads to tetraacetates which are shown, by a method of distinguishing between N-acetyl and O-acetyl groups, to have all of the acetyl groups esterified with hydroxyls. The method depends upon the stability of N-acetyl groups to alkaline conditions under which O-acetyl groups are removed.[137] Since all acetyl groups are esterified with hydroxyl groups, the tetraacetyl glucose and galactose phenylosazones must be open-chain compounds, for the presence of a ring would allow only a triacetate to be formed. It should be noted, however, that this method may not always be relied upon. For example, α,β-diacetyl-phenylhydrazine gives up one acetyl group under the conditions of the O-acetyl determination. (See also p. 411.)

A comparison of the absorption curves of the sugar osazones with those of simple substances[115] indicates that the sugar osazones are acyclic but methylation studies[138] show the presence of a ring structure as illustrated in the following series of reactions:

$$
\text{Glucosazone} \xrightarrow[\text{NaOH}]{(CH_3)_2SO_4} \text{trimethylglucosazone} \xrightarrow{p\text{-nitrobenzaldehyde}}
$$

$$
\text{trimethylglucosone} \xrightarrow[\text{HOAc}]{\text{Zn}} 3,4,5\text{-trimethylfructopyranose}
$$
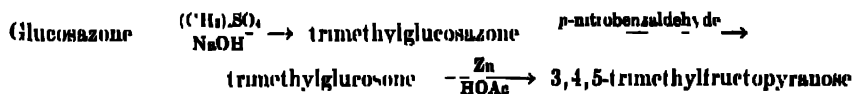
Since the hydroxyl of carbon 6 is not methylated, it is probably involved in ring formation. Inasmuch as the methylation of the osazones proceeds with difficulty and most of the products are amorphous, this evidence cannot be considered as final, although the analogous behavior of the osazones, hydrazones and other nitrogenous derivatives makes a ring structure seem probable. Similar methylation evidence indicates a ring structure for galactosazone.[139]

[135] E. E. and E. G. V. Percival, J. Chem. Soc., 750 (1941); E. Votoček and R. Vondráček, Ber., 37, 3848 (1904); C. Neuberg, Ber., 32, 3387 (1899).

[147] M. L. Wolfrom, M. Konigsberg and S. Soltzberg, J. Am. Chem. Soc., 58, 490 (1936).

[138] E. E. and E G. V. Percival, J. Chem. Soc., 1398 (1935).

[139] J. R. Muir and E G V. Percival, J. Chem. Soc., 1479 (1940).

The osazones of the sugars are converted to osotriazoles when they are heated in aqueous copper sulfate solution.[140] These derivatives offer considerable promise for the identification of the sugars and as confirmatory tests for the presence of the parent osazones. The opportunity for isomerism is less than for the osazones; hence, the melting points and optical rotations are of greater value for identification purposes.

Osotriazoles of diketones previously had been described by v. Pechmann[141] who obtained them by the oxidation of the corresponding dihydrazones. The preparation of the osotriazole of diacetyl is illustrated below:

$$
\begin{array}{c}
\text{CH}_3 \\
|\\
\text{C}=\text{NNHC}_6\text{H}_5 \\
|\\
\text{C}=\text{NNHC}_6\text{H}_5 \\
|\\
\text{CH}_3
\end{array}
\xrightarrow{\text{K}_3\text{FeO}}
\begin{array}{c}
\text{CH}_3 \\
|\\
\text{C}=\text{NNC}_6\text{H}_5 \\
|\quad|\\
\text{C}=\text{NNC}_6\text{H}_5 \\
|\\
\text{CH}_3
\end{array}
\xrightarrow{\text{HCl}}
$$

Diacetyl   Diacetyl
diphenylhydrazone   osotetrazone

$$
\begin{array}{c}
\text{CH}_3 \\
|\\
\text{C}=\text{N} \\
|\qquad\diagdown \\
\qquad\qquad \text{NC}_6\text{H}_5 \\
|\qquad\diagup \\
\text{C}=\text{N} \\
|\\
\text{CH}_3
\end{array}
\quad + \quad \text{C}_6\text{H}_5\text{NH}
$$

Diacetyl
osotriazole
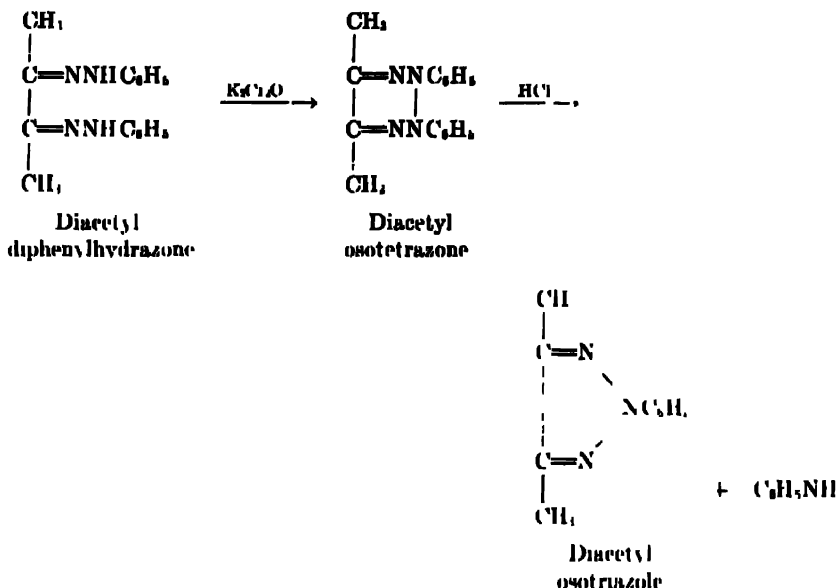
The corresponding osotriazoles of the sugars are formed directly by the catalytic action of copper sulfate. The formation of the phenyl-D-glucotriazole (II) from glucose phenylosazone (I) is illustrated below. Its structure is demonstrated by oxidation with periodic acid to the 2-phenyl-4-formyl-osotriazole (III) which is identical with the product obtained previously by v. Pechmann from monoacetyldinitrosoacetone phenylhydrazone (IV).

Reduction of glucose phenylosazone by zinc and acetic acid[142] or by catalytic hydrogenation[143] leads to the complete removal of one group,

[140] R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **66**, 735 (1944).
[141] H. v. Pechmann, *Ber.*, **21**, 2751 (1888); *Ann.*, **262**, 265 (1891).
[142] E. Fischer, *Ber.*, **19**, 1920 (1886).
[143] K. Maurer and B. Schiedt, *Ber.*, **68**, 2187 (1935).

$$
\begin{array}{llll}
\text{HC}=\text{NNHC}_6\text{H}_5 & \text{HC}=\text{N} & \text{HC}=\text{N} & \text{HC}=\text{N(OAc)} \\
| & |\quad\diagdown\text{NC}_6\text{H}_5 & |\quad\diagdown\text{NC}_6\text{H}_5 & | \\
\text{C}=\text{NNHC}_6\text{H}_5 & \text{C}=\text{N}\diagup & \text{C}=\text{N}\diagup & \text{C}=\text{NNHC}_6\text{H}_5 \\
| & | & |\qquad\leftarrow - & | \\
\text{HOCH} & \text{HOCH} & \text{HCO} & \text{HC}=\text{NOH} \\
| & | & + & \\
\text{HCOH} & \text{HCOH} & \text{HCHO} & \\
| & | & + & \\
\text{HCOH} & \text{HCOH} & & \\
| & | & 2\ \text{HCOOH} & \\
\text{H}_2\text{COH} & \text{H}_2\text{COH} & & \\
\text{(I)} & \text{(II)} & \text{(III)} & \text{(IV)}
\end{array}
$$

(with CuSO_4 over the arrow between I and II; $-\text{HIO}_4$ over the arrow between II and III)
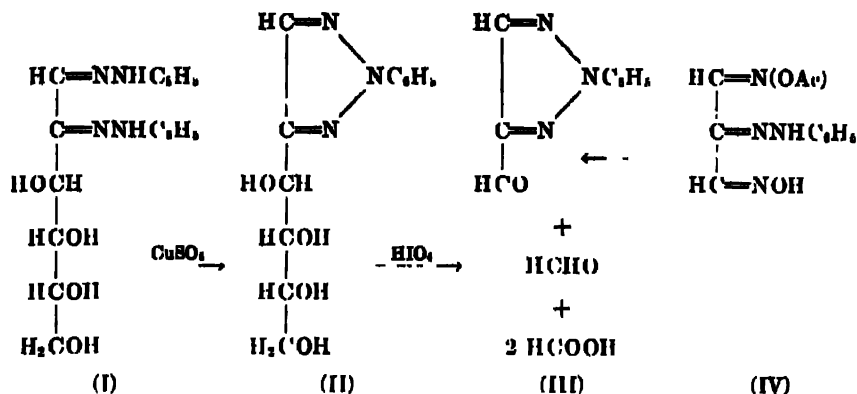
the splitting of the other and the formation of 1-desoxy-1-aminofructose. The aminofructose often is called isoglucosamine because it has the same empirical formula as glucosamine. The structure of the amine is shown by its reaction with nitrous acid to produce D-fructose.[144] Similar derivatives, with a substituted amino group, result through the Amadori rearrangement of the corresponding N-glucosides as described earlier in this chapter.

When the acetyl groups of acetylated sugar osazones are removed by the use of sodium hydroxide, anhydro derivatives are formed. Percival[145] has shown that the phenylosazones of the tetraacetates of glucose, galactose and gulose yield the same dianhydrohexose phenylosazone and, hence, that the anhydro rings must involve carbons 3 and 4. This conclusion must be correct, for the three sugars differ only in the configurations of these two carbons. Evidently, Walden inversion must take place in the formation of the anhydro ring for certain of the sugars but not for all. The structure (I) is confirmed by the inability of the compound to yield a trityl derivative (no CH_2OH group) and by the formation of a monotosyl derivative (one free hydroxyl). The configuration of the asymmetric carbon atoms has not been demonstrated.

$$
\begin{array}{l}
\text{CH}=\text{N}-\!\!\!\rceil \\
\quad | \\
\ulcorner\!-\text{C}-\text{NH} \\
|\qquad | \\
|\ \ \text{HC}\diagdown\ \text{NPh} \\
\text{O}\quad |\quad\diagup \\
|\ \ \text{HC}-\text{N}-\text{Ph}\qquad\text{(I)} \\
|\qquad | \\
\quad\text{HCOH} \\
\lfloor\!-\text{CH}_2
\end{array}
$$
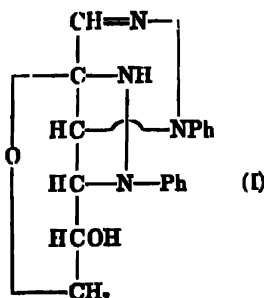
[144] E. Fischer and J. Tafel, *Ber.*, **20**, 2566 (1887).
[145] E. G. V. Percival, *J. Chem. Soc.*, 1384 (1933).

Monoanhydro derivatives of glucosazone, galactosazone, xylosazone, arab-inosazone, cellobiosazone and lactosazone and a dianhydromaltosazone have been made by boiling alcoholic solutions of the osazones with a little sulfuric acid.[146] Percival[147] has assigned the following structure to the monoanhydroglucosazone:

$$
\begin{array}{c}
\text{┌──────O──────┐} \\
\text{│   H OH   │} \\
\text{│   │ │   │} \\
\text{CH}_2\text{ -CH--C--C -C   CH=NNHPh} \\
\text{│   │ │} \\
\text{│   OH H} \\
\text{PhN ------- NH}
\end{array}
$$
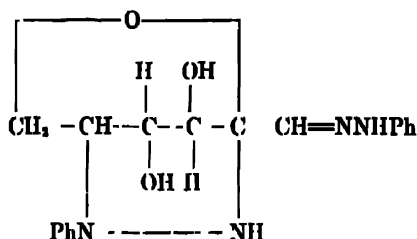
The solubility characteristics of the reaction products of the sugars with unsubstituted *hydrazine* (NH_2—NH_2) are not favorable for identification purposes. The aldoses form aldazines, and the ketoses ketazines, in which two moles of the sugar are combined with one mole of the hydrazine.[148a] However, hydrazine reacts readily with sugar lactones to give characteristic derivatives useful for identification. The lactones may be regenerated from the hydrazides by treatment with nitrous anhydride.[148b]

B. Oximes. The sugars, probably in the free-aldehyde form (I), react[149] with hydroxylamine to give the sugar oximes (II or III).

$$
\begin{array}{ccccc}
HC\!\!=\!\!O & & HC\!\!=\!\!N \quad OH & & HC\text{ -NOH} \\
| & \xrightarrow[\text{KOAc}]{\text{NH}_2\text{OH HCl}} & | & \rightleftharpoons & | \\
HCOH & & HCOH & & H \\
| & & | & & HCOH \quad O \\
& & & & | \\
(I) & & (II) & & (III)
\end{array}
$$
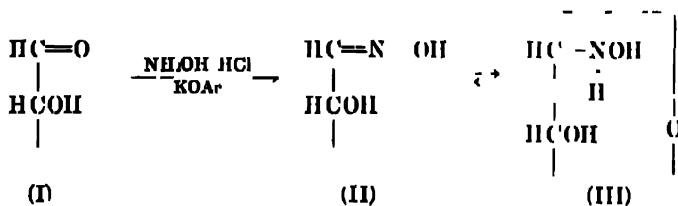
The oximes are too soluble in water and in alcohols to be of general value for the identification of the sugars, but they are very useful for preparing acyclic derivatives and for shortening the carbon chains of the sugars (Wohl degradation).

Since the oximes mutarotate,[150] the simple structure II is not sufficient

[146] O. Diehls and R. Meyer, *Ann.*, *519*, 157 (1935); E. Fischer, *Ber.*, *17*, 579 (1884); *20*, 821 (1887).

[147] E. G. V. Percival, *J. Chem. Soc.*, 783 (1945).

[148a] F. Davidis, *Ber.*, *29*, 2308 (1896).

[148b] A. Thompson and M. L. Wolfrom, *J. Am. Chem. Soc*, *68*, 1509 (1946).

[149] P. Rischbieth, *Ber*, *20*, 2673 (1887); E. Fischer and J. Hirschberger, *Ber.*, *22*, 1155 (1889).

[150] H. Jacobi, *Ber.*, *24*, 696 (1891).

unless *syn* and *anti* isomers exist. By analogy with the sugars, the mutarotation may be the result of the establishment of an equilibrium between the open chain (II) and cyclic isomers (III). Although only one crystalline glucose oxime is known, two crystalline hexaacetates have been isolated.[161] One is obtained by the reaction of *aldehydo*-pentaacetylglucose with hydroxylamine followed by acetylation. Because it is prepared from the acyclic form of glucose, it must be the acyclic oxime. At low temperatures, acetylation of glucose oxime produces a second hexaacetate.[163] This second isomer probably is a ring modification because crystalline 2,3,4,6-tetramethylglucose is produced after methylation and hydrolysis.[162] Confirmation of these structures for the hexaacetylglucose oximes is given by a method which distinguishes between N-acetyl (or N-acetoxy) and O-acetyl groups. As would be expected, the cyclic modification has an N-acetoxy group, and the acyclic hexaacetate has only O-acetyl groups.[161] This method depends upon the stability of the N-acetoxy group in alkaline solution. Thus, the acid-hydrolysis procedure of Freudenberg and Harder[165] removes all the acetyl groups of both hexaacetylglucose oximes, but alkaline hydrolysis removes only five O-acetyl groups from the cyclic form and all six from the open-chain form. This resistance of N-acetyl and N-acetoxy groups to alkaline hydrolysis also seems to exist for other nitrogenous derivatives of the sugars. (See, however, p 407.)

In solution, as is evidenced by the mutarotation and other reactions of the oximes, the cyclic and acyclic modifications of the sugar oximes seem to be in equilibrium Deacetylation of the acyclic hexaacetylglucose oxime leads to the known, cyclic, crystalline glucose oxime. This isomerization is additional proof for an equilibrium between the various forms. Studies of the acetylation of the sugar oximes furnish additional proof.[161, 164] Low temperature acetylation of glucose oxime (I) with acetic anhydride and pyridine produces the acetylated cyclic isomer (V); at higher temperatures, the gluconitrile pentaacetate (II) is the main product. Since at the higher temperatures the acyclic hexaacetylglucose oxime (III) gives good yields of the gluconitrile pentaacetate (II), the acyclic oxime (IV) is probably an intermediate in the preparation of the nitrile. Thus, both cyclic and acyclic acetates are formed in the acetylation reaction.

The products obtained upon acetylation of the sugar oximes depend not only on the temperature but also on the configuration of the sugar in-

[161] M L. Wolfrom and A. Thompson, *J Am. Chem. Soc.*, **53**, 622 (1931); A. Wohl, *Ber.*, **26**, 730 (1893).

[162] J. C. Irvine and R. Gilmour, *J. Chem. Soc*, **93**, 1429 (1908).

[163] R. Behrend, *Ann.*, **353**, 106 (1907).

[164] M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *J. Am. Chem. Soc.*, **58**, 490 (1936).

[165] K. Freudenberg and M Harder, *Ann.*, **433**, 230 (1923).

$$
\begin{array}{ccccccc}
\text{HC--NOH} & & \text{HC=NOH} & & \text{CN} & & \text{HC=NOAc} \\
\overset{\centerdot}{\text{H}}\quad\text{O} & \xrightarrow[\text{heat}]{(Ac)_2O} & \text{HCOAc} & \to & \text{HCOAc} & \xrightarrow[\text{heat}]{(Ac)_2O} & \text{HCOAc} \\
\text{HCOH} & & & & & & \\
\text{(I)} & & \text{(IV)} & & \text{(II)} & & \text{(III)}
\end{array}
$$

$$
\Big\downarrow \begin{array}{c}(Ac)_2O \\ \text{cold}\end{array}
$$

$$
\begin{array}{c}
\text{HC---NOAc} \\
\overset{\centerdot}{\text{Ac}}\quad\text{O} \\
\text{HCOAc}
\end{array}
$$

(V)
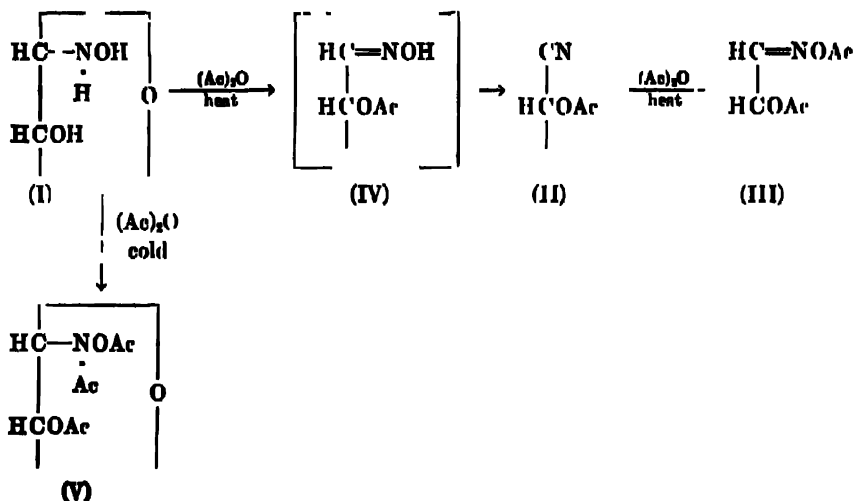
volved.[154] The arabinose, rhamnose, xylose and glucosamine oximes yield only the nitriles as a result of low-temperature acetylation; glucose gives the cyclic hexaacetate and mannose and fucose the acyclic hexaacetates; galactose yields a mixture of all three types. At higher temperatures, the proportion of the nitrile in the reaction mixture increases considerably.

The Wohl method for shortening the carbon chain of the sugars utilizes the acetylated nitrile prepared by the above procedure and is described in more detail elsewhere (Chapter III).

5. Derivatives in Which an Amino Group Replaces a Primary or Secondary Hydroxyl Group

A. Amino sugars (Glycosamines).[157] *Occurrence and Structure.* Sugar derivatives which have an amino group in place of one of the primary or secondary hydroxyls of the sugars comprise the amino sugars. The glycosylamines (osimines) have been considered separately on page 376 since their amino group is much more labile than that in the stable amino sugars. These compounds are of considerable interest because several are found among the hydrolytic products of many polysaccharides and glycoproteins. All of the natural members of this group are 2-desoxy-2-amino-aldoses. Chitin, the principal polysaccharide of fungi, insects and crustacea, gives 2-desoxy-2-aminoglucose (glucosamine or chitosamine) on hydrolysis.[158] A second naturally occurring aminohexose is chondrosamine

[154] R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc*., **59**, 1898 (1937); E. Restelli de Labriola and V. Deulofeu, *ibid*., **62**, 1611 (1940).

[157] P. A. Levene, *Biochem Z*, **124**, 37 (1921); *J. Biol. Chem*., **63**, 95 (1925); "Hexosamines and Mucoproteins"; Longmans, Green and Co., London (1925).

[158] G. Ledderhose, *Z. physiol. Chem*., **2**, 213 (1878); **4**, 139 (1880).

which forms galactosazone on treatment with phenylhydrazine;[157,158] it is 2-desoxy-2-aminogalactose also known as galactosamine (see below).

The N-methyl derivative of L-glucosamine (the enantiomorph of the common D-glucosamine) has been isolated from the degradation product of streptomycin.[160] It is interesting to speculate that the antibiotic activity of the streptomycin may arise from the presence of the L-form of the glucosamine.

The structure of glucosamine is shown by the following reactions. The reducing group is unsubstituted[161] since the compound reduces Fehling solution and is oxidized by bromine to a six-carbon acid (2-desoxy-2-amino-gluconic acid, glucosaminic acid). The reaction with phenylhydrazine takes place with the loss of the amino group and the formation of glucosazone.[162] This evidence locates the amino group at position 2, but the compound might be related configurationally to either glucose or mannose. Research has been conducted on this problem but has proved difficult because of lack of knowledge concerning the mechanism of the removal of the amino group and of the mechanism of the reactions involved in the synthesis of the compound. Since both glucose and mannose derivatives may be obtained from glucosamine, a Walden inversion must be involved in at least one of the reactions. The information obtained from the hydrolysis of the tosyl and the anhydro derivatives of the sugars, however, has made it possible to predict the occurrence of Walden inversions, and glucosamine has been shown to be a derivative of glucose and not of mannose.

The final evidence[164] is provided by the synthesis of a glucosamine derivative (I) (accompanied by a derivative of 3-desoxy-3-aminoaltrose) by the reaction of ammonia on methyl 2,3-anhydro-4,6-dimethyl-β-mannoside (II). Inasmuch as ammonia reacts in the same manner with anhydro rings as does sodium methylate and inasmuch as the same anhydro-mannoside reacts with sodium methylate with the formation of glucose (III) and altrose derivatives, the glucosamine in all probability has the glucose configuration. Application of Hudson's isorotation principle also leads to a correlation with the glucose instead of the mannose configuration.[165]

[159] P. A. Levene and F. B. LaForge, J. Biol. Chem., 18, 123 (1914).
[160] F. A. Kuehl, Jr., and Associates, J. Am. Chem. Soc., 68, 536 (1946).
[161] E. Fischer and F. Tiemann, Ber., 27, 138 (1894).
[162] F. Tiemann, Ber., 19, 49 (1886).
[163] Most of the previous work has also shown that the substance has the glucose structure; the evidence has been reviewed by S. Peat, Ann. Repts on Progress Chem. (Chem. Soc. London)., 34, 289 (1937).
[164] W. N. Haworth, W. H. G. Lake and S. Peat, J. Chem. Soc., 271 (1939).
[165] A Neuberger and R. P Rivers, J. Chem. Soc , 122 (1939).

Methyl 2,4,6-
trimethyl-
β-glucoside

Methyl 2,3-
anhydro-4,6-
dimethyl-
β-mannoside

Methyl 2-desoxy-
2-amino-4,6-di-
methyl-β-gluco-
side (methyl 4,6-
dimethyl-β-
glucosaminide)

(III)   (II)   (I)
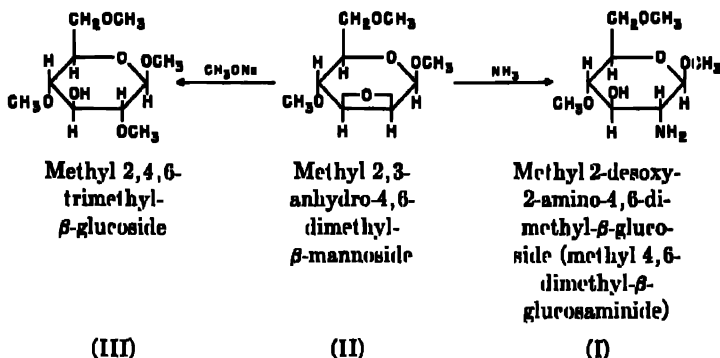
Chondrosamine has been synthesized by methods that fix its configuration as 2-desoxy-2-aminogalactose.[166] The synthesis has been accomplished by the ammonolysis of 1,6-2,3-dianhydrotalose (IV). The ammonia adds to the 2,3-anhydro ring, and subsequently the 1,6-anhydro ring is cleaved by acid hydrolysis. One of the two products obtained was shown to be identical with natural chondrosamine. Since the addition of ammonia to the anhydro ring very probably takes place with Walden inversion at the carbon atom to which the amino group becomes attached, the natural material (V) must be a galactose derivative and the other a 3-desoxy-3-amino-idose derivative (VI).

(IV)   (V)   (VI)
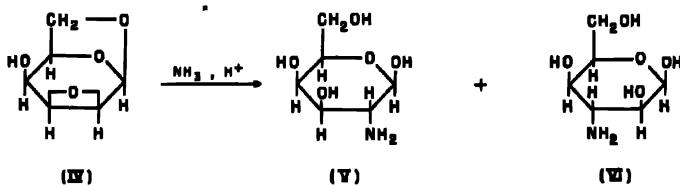
Proof for the configuration of VI is given by its synthesis (along with 4-desoxy-4-aminomannose) from 1,6-3,4-dianhydrotalose by ammonolysis and hydrolysis as above.

Anhydro Derivatives from Glucosamine. Treatment of glucosamine with nitrous acid does not lead to the replacement of an amine by a hydroxyl group; instead an anhydro ring is formed.[166,167] The anhydrosugar formed, called chitose (I), is a 2,5-anhydrosugar, for oxidation leads to chitonic acid (II) which is converted by the action of acetic anhydride to 5-hydroxy-methylfuroic acid (III) of known structure.

When the above order of operations is reversed, *i.e.*, when the oxidation precedes the treatment with nitrous acid, chitaric acid is formed. This

[166] S. P. James, F. Smith, M. Stacey and L. F. Wiggins, *Nature*, *156*, 309 (1945).
[167] E. Fischer and E. Andreae, *Ber.*, *36*, 2587 (1903).

anhydroaldonic acid is isomeric with chitonic acid. (For additional details, see under Anhydro Derivatives, p. 356.)

$$\begin{array}{c} HCO \\ | \\ HCNH_2 \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ H_2COH \end{array} \xrightarrow{HONO} CH_2OH-CH \underset{O}{\overset{CH(OH)-CH(OH)}{\diagdown\diagup}} CH-CHO \xrightarrow{Br_2}$$

(I)

$$CH_2OH-CH \underset{O}{\overset{CH(OH)-CH(OH)}{\diagdown\diagup}} CH-COOH \xrightarrow{(Ac)_2O} CH_2OH-C \underset{O}{\overset{CH---CH}{\diagdown\diagup}} C-COOH$$

(II)   (III)
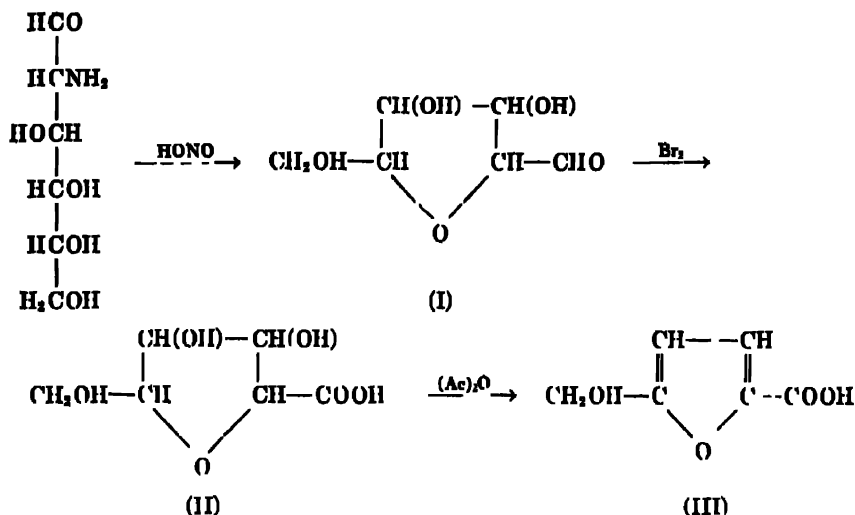
Preparation and Synthesis of Amino Sugars. Lobster and crab shells, which consist of calcium carbonate, chitin and protein material, are hydrolyzed by concentrated hydrochloric acid to yield glucosamine and provide good sources of this substance.[168,169] Glucosamine, obtained as the hydrochloride, is converted to the free base by treatment with diethylamine or sodium methylate. The mycelium of the lower fungi has also been utilized for the preparation of glucosamine.[169] Galactosamine (chondrosamine) is formed from the naturally occurring chondroitin sulfuric acid (e.g., from cartilage and nasal septa) by the action of hydrochloric acid and zinc chloride.[170]

The difficultly soluble N-carbobenzoxyglucosamine, prepared by the action of carbobenzoxy chloride ($C_6H_5CH_2OCOCl$) on glucosamine, has been suggested[171] for the separation of the amino sugar from accompanying sugars. Schiff bases, particularly those from 2-hydroxy-1-naphthaldehyde, are of value for the isolation of both glucosamine and chondrosamine.[172]

The classical method for the synthesis of 2-amino sugars of the natural type utilizes the osimines obtained by the action of ammonia on sugars.[170,173] The process, which involves the addition of hydrogen cyanide to the osimines, results in the lengthening of the carbon chain; hence, D-arabino-

[168] C. S. Hudson and J. K. Dale, *J. Am. Chem. Soc.*, 38, 1431 (1916).
[169] See: O. E. May and G. E. Ward, *J Am. Chem. Soc.*, 56, 1597 (1934).
[170] P. A. Levene, *J. Biol. Chem.*, 26, 147 (1916).
[171] E. Chargaff and M. Bovarnick, *J. Biol. Chem.*, 118, 421 (1937)
[172] Z. E. Jolles and W. T. J. Morgan, *Biochem. J.*, 34, 1183 (1940).
[173] E. Fischer and H. Leuchs, *Ber.*, 36, 24 (1903).

simine must be used for obtaining glucosamine. The creation of a new asymmetric carbon results in the formation of two epimeric nitriles which are hydrolyzed to the corresponding acids and then reduced to the corre-
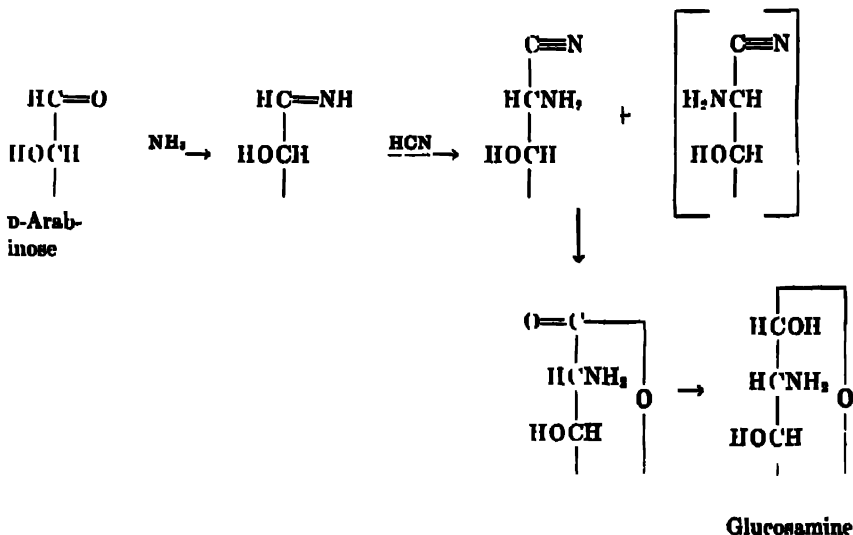
Glucosamine

sponding sugar amines. The glucosamine prepared in this fashion is identical with the natural product. The isomer obtained from the second nitrile, 2-mannosamine, is the true epiglucosamine. A second so-called "epiglucosamine," prepared by the following sequence of reactions,[174] has been shown to be 3-desoxy-3-aminoaltrose.[175]

Glucal $\xrightarrow{Br_2}$ 1,2-dibromoglucal $\xrightarrow[Ag_2CO_3]{CH_3OH}$ methyl 2-bromoglucoside $\xrightarrow{NH_3}$

methyl 3-desoxy-3-aminoaltroside ("methyl epiglucosaminide").
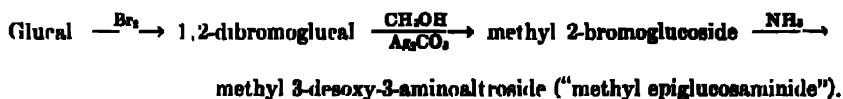
This transformation from a glucose to an altrose derivative probably takes place through the intermediate formation a of 2,3-anhydro derivative, as originally suggested by Fischer, Bergmann and Schotte,[174] and involves several Walden inversions. Evidence for this mechanism is supplied by the synthesis (considered above in connection with the discussion of the configuration of glucosamine) of 3-desoxy-3-aminoaltrose and 2-desoxy-2-aminoglucose derivatives from methyl 2,3-anhydro-β-mannoside and ammonia. The preparation of amino sugars from the glycals is of particular value when amino derivatives of the rarer sugars are desired, for Walden

[174] E. Fischer, M. Bergmann and H. Schotte, *Ber.*, **53**, 509 (1920).

[175] W. N. Haworth, W. H. G. Lake and S. Peat, *J. Chem. Soc.*, 271 (1939).

inversions take place during the formation of the anhydro rings and during the opening of the rings by ammonia.

The preparation of the 6-amino derivatives involves the action of ammonia on the 6-halogeno,[176] the 6-tosyl or the 5,6-anhydro derivatives[177] of the sugars.

As described previously, glucosazone may be reduced to 1-desoxy-1-aminofructose (isoglucosamine). The 1-(arylamino)-fructoses also are formed by the action of dilute acids on the N-glucosides (see Amadori rearrangement).

Reactions of the Amino Sugars. The amino groups as well as the hydroxyl groups are esterified when the usual methods for acetylation are employed, and alpha and beta isomers are produced. The N-acetyl group is more stable than O-acetyl groups, and, by alkaline hydrolysis of the fully acetylated derivative, N-acetylglucosamine is obtained. The reaction of the sugar amines with aldehydes leads to Schiff bases. Those formed from 2-hydroxy-1-naphthaldehyde are valuable for the separation of small quantities of glucosamine and chrondosamine.[172] That from *p*-methoxybenzaldehyde has value for obtaining the tetraacetylglucosamine in which the amino group is not acetylated.[178] Thus, the Schiff base (I) is acetylated; the aldehyde residue then is removed by acids to give 1,3,4,6-tetraacetylglucosamine (II). Acyl derivatives of the amino sugars in which the acylating substance is an amino acid or polypeptide have been studied (see later section of this chapter).

HCOH
|
HC —NH₂   O   →   HC—N=CHR   O   →
|
HOCH   HOCH

(R= p-CH₃O—C₆H₄—)
(I)

HCOAc   HCOAc
| |
HC—N=CHR   O   →(HCl)→   HC—NH₂   O
| |
AcOCH   AcOCH

(II)
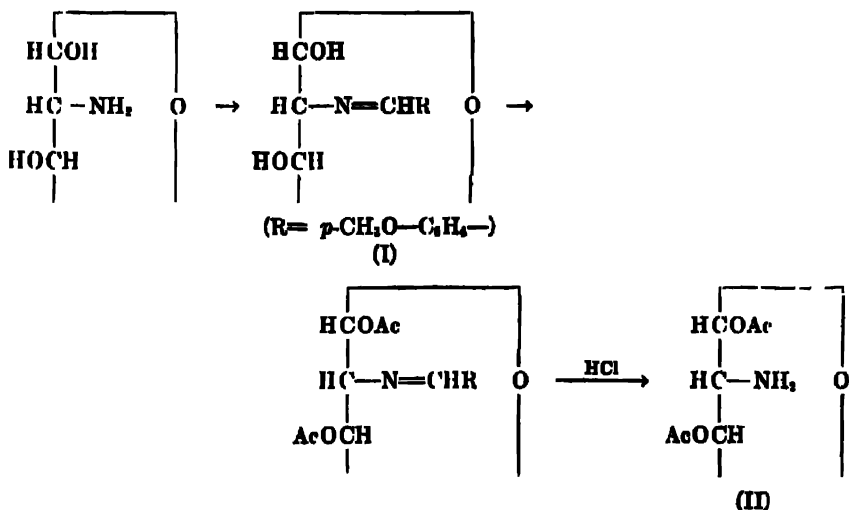
[176] E. Fischer and K. Zach, *Ber.*, *44*, 132 (1911).
[177] H. Ohle and associates, *Ber.*, *61*, 1203 (1928); *69*, 1022, 1636, 2311 (1936).
[178] M. Bergmann and L. Zervas, *Ber.*, *64*, 975 (1931).

The amino sugars form hydrazones, oximes, acyl halides, glycosides, and benzylidene derivatives analogous to those of the sugars. The methyl glycoside (methyl glucosaminide) is very resistant to acid hydrolysis and has been considered to have a betaine structure:[179]

$$
O \left[\begin{array}{l} \text{HC}-\text{O}^- \\ \text{HC}-\overset{+}{\text{NH}}_2-\text{CH}_3 \end{array} \right.
$$
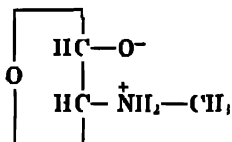
A betaine structure is also supported by the evolution of methylamine when the compound is treated with alkali. However, the above formula seems improbable and the formation of the methylamine is explained better by a combination of formaldehyde and ammonia formed in the alkaline decomposition of the methyl 2-desoxy-2-aminoglucoside, the resistance to acid hydrolysis is probably due to the action of the positively charged amino group in repelling the hydrogen ion as it approaches the linkage undergoing hydrolysis.[180] The value for the activation energy of the hydrolytic reaction agrees with this concept, for it is similar to that of the ordinary glycosides. The pyranose ring structure of the methyl 2-desoxy-2-aminoglucoside is shown by the following reactions.[179,181]

Methyl 2-desoxy-2-aminoglucoside $\xrightarrow[\text{CH}_3\text{I}]{\text{Ag}_2\text{O}}$

methyl 2 desoxy 2-(dimethylamino)-glucoside $-\xrightarrow{\text{Ba(OH)}_2}$

methyl glucoside (not isolated) $- \rightarrow$

methyl tetramethylglucoside $\xrightarrow{\text{H}^+}$ 2,3,4,6 tetramethylglucose
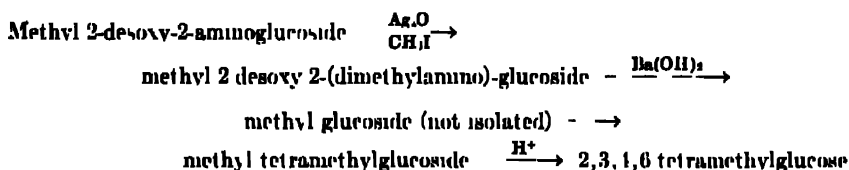
The phenyl 2-desoxy-2-(acetylamino)-glucosides are hydrolyzed by almond emulsin, but the enzyme (β-glucosaminidase) differs from the β-glucosidase which hydrolyzes the β-glucosides.[182] Snail emulsin from *Helix pomatia* has also been shown to have different enzymes for the hydrolysis of glucosides and 2-desoxy-2-N-acetylaminoglucosides.[183]

Glucosamine may be estimated by the usual iodine titration or by copper-reduction methods. A procedure based on the production of a color by the reaction of alkali-treated N-acetylglucosamine with the Ehrlich reagent (p-dimethylaminobenzaldehyde in hydrochloric acid) has been devised for the determination of glucosamine.[184] The reagent gives a red color with

[179] J. C. Irvine and A. Hynd, *J. Chem. Soc*, **101**, 1128 (1912).

[180] R. C. G. Moggridge and A. Neuberger, *J. Chem. Soc.*, 745 (1938).

[181] A. Neuberger, *J. Chem Soc.*, 29 (1940).

[182] B. Helferich and A. Iloff, *Z. physiol. Chem.*, **321**, 252 (1933).

[183] A Neuberger and R. V. P. Rivers, *Biochem. J.*, **33**, 1580 (1939).

[184] F. Zuckerkandl and L. Messiner-Klebermass, *Biochem. Z.*, **236**, 19 (1931); W. T. J Morgan and L. A. Elson, *Biochem. J.*, **28**, 988 (1934).

pyrroles, indicating that a heterocyclic ring may be formed by the action of alkali on N-acetylglucosamine. The structure of the product formed by the action of alkali on N-acetylglucosamine has been investigated by White[185] who concludes that a glucoxazoline is formed.

$$CH_2OH—CH—(CHOH)_2—CH—CH———$$

$$\begin{array}{c} | \\ O \\ | \\ N\!\!=\!\!\!=\!\!C—CH_3 \end{array}$$

2-Methyl-4,5-glucopyrano-
—Δ^2-oxazoline
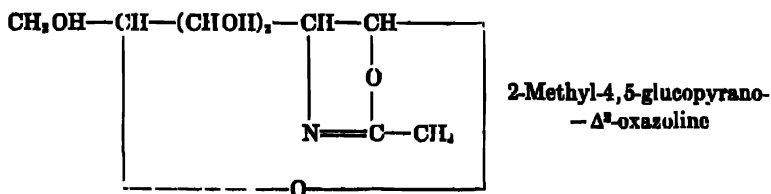
B. Glycamines and Desoxy Amino Glykitols. Derivatives of sugar alcohols in which a CH_2OH group has been replaced by a CH_2NH_2 or CH_2NHR group are known as glycamines. The systematic name would be 1-desoxy-1-amino glykitols. The first of these derivatives was prepared by Maquenne and Roux[186] by the reduction of oximes. Because vitamin B_2 (riboflavin) is a derivative of D-ribamine (1-desoxy-1-aminoribitol), the group of glycamines holds considerable interest for the biochemist. Glucamine (1-desoxy-1-aminosorbitol) and methylglucamine (1-desoxy-1-methylamino-sorbitol) appear to show some promise as intermediates for the preparation of wetting agents[187] and as solubilizing groups for pharmaceuticals such as theophylline.[188]

The lower homologs of this series include ethanolamine ($NH_2CH_2CH_2OH$) and 2,3-dihydroxy-n-propylamine. Ethanolamine in particular has achieved considerable industrial importance.

As described earlier, the glycosylamines (I) may be reduced by catalytic hydrogenation to glycamines (II).[189]

$$\begin{array}{ccc} HC\!\!=\!\!NR & & H_2C—NHR \\ | & \xrightarrow[\text{H.}]{\text{Ni}} & | \\ HCOH & & HCOH \\ | & & | \\ (I) & & (II) \end{array}$$
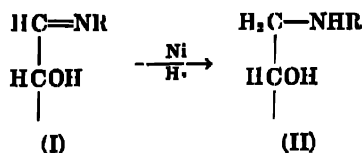
[185] T. White, *J. Chem. Soc.*, 428 (1940); W. H. Bromund and R. M. Herbst, *J. Org Chem.*, *10*, 267 (1945).

[186] L. Maquenne and E. Roux, *Compt. rend.*, *132*, 980 (1901); E. Roux, *Ann. chim. phys.*, [8] *1*, 72 (1904).

[187] See for example: W. S. Calcott, U. S. Patent 2,060,850, Nov. 17, 1936; 2,016,956, Oct. 8, 1935; H. A. Piggot, U. S. Patent, 1,985,424, Dec. 25, 1934; 2,091,105, Aug. 24, 1937.

[188] E. H. Volwiler and E. E. Moore, U. S. Patent 2,161,114, June 6, 1939.

[189] See references previously given and: R. B. Flint and P. L. Salzberg, U. S. Patent 2,016,962-3, Oct. 8, 1935; P. L. Salzberg, U. S. Patent 2,193,433, Mar. 12, 1940.

The Amadori rearrangement of glucosylarylamines gives 1-arylamino-fructoses, which may be reduced to glucamines and mannamines. (See under Amadori rearrangement). Reduction of glucosazone with sodium amalgam gives fructosamine (1-desoxy-1-aminofructose).[190]

Closely related derivatives are produced by the reduction of glucosamine and derivatives to a product sometimes known as glucosaminol. A more systematic name is 2-desoxy-2-aminosorbitol. The N-acetyl derivative is obtained by the catalytic hydrogenation of N-acetylglucosamine and the unacetylated compound by reduction of the hydrochloride.[191] Free glucosamine undergoes an interesting Cannizzaro reaction when reduced catalytically to give 2-desoxy-2-aminogluconic acid (glucosaminic acid) and 2-desoxy-2-aminosorbitol.[192]

$$
\begin{array}{ccccc}
\text{HC=O} & & \text{CH}_2\text{OH} & & \text{COOH} \\
| & & | & & | \\
? \quad \text{HCNH}_2 & \xrightarrow[\text{Pt}]{\text{H}_2} & \text{HCNH} & + & \text{HCNH}_2 \\
| & & | & & | \\
\text{HOCH} & & \text{HOCH} & & \text{HOCH}
\end{array}
$$
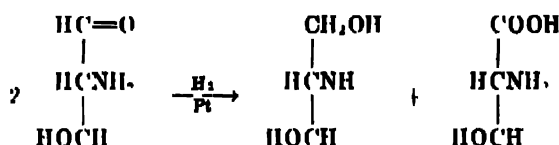
The nitrogen atom of glucamines may be used to form salts with fatty acids that are said to be of value as wetting agents and for other purposes (See above). With oxalic acid, they form characteristic salts.

Nitro alcohols corresponding to the glycamines are obtained by treatment of sugars with nitromethane.[193] The 2,4-benzylidene-L-xylopyranose in methanol solution adds nitromethane under the influence of sodium methylate to give 2,4-benzylidene-6-nitro-6-desoxy-D-sorbitol from which the benzylidene group is removed by hydrolysis with acids. Catalytic reduction gives the corresponding amino alcohols.

6. Combinations of Sugars with Amino Acids and Proteins[194]

Colorimetric methods indicate that most proteins contain several per cent of carbohydrates.[195] The carbohydrate portion, although small, is of considerable biological importance. Many such combinations act as antigens

[190] E. Fischer, *Ber.*, *19*, 1920 (1886).

[191] P. Karrer and J. Meyer, *Helv. Chim. Acta*, *20*, 626 (1937).

[192] P. A. Levene and C. C. Christman, *J. Biol. Chem.*, *130*, 575 (1937).

[193] J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, *67*, 1713 (1945); *68*, 1511 (1946).

[194] For early history see: S. Fränkel and C. Jellinek, *Biochem. Z.*, *185*, 392 (1927).

[195] M. Sørensen and G. Haugaard, *Biochem. Z.*, *260*, 247 (1933); S. Gurin and D. B. Hood, *J. Biol. Chem.*, *139*, 775 (1941).

and induce the formation of antibodies in animals, and often the specificity is due mainly to the carbohydrate portion. It has been suggested that the enzymes which hydrolyze carbohydrates (glycosidases) may be proteins which contain carbohydrates and that the sugar portion may be responsible for the marked specificity shown by these enzymes.[196]

The combinations of amino acids with sugars may play an important part in the changes which take place during the dehydration and storage of natural products. As shown by the early researches of Maillard and others,[197] solutions of sugars and amino acids develop brown to black colors and pronounced odors when heated. The development of these changes may be detrimental in many foods such as in dried fruits and eggs. On the other hand, they may be beneficial as in malt, for the color, odor and foaming properties impart desirable characteristics to beer.

A. Preparation. The relationship of condensation products of sugars and amino acids to labile complexes of carbohydrates and amino acids and to the melanoidin reaction has stimulated the study of the simplest systems. The amino acids may condense with the aldehyde group of sugars in a manner similar to that of amines:

$$\text{HCO} \quad + \quad \text{R--CH--COOH} \quad \rightarrow \quad \overset{\text{R}}{\underset{\text{HCOH}}{\text{HC}=\text{N--CH}}}$$

HCO   R --CH—COOH   HC=N--CH
 | | | |
HCOH   NH₂   HCOH   COOH
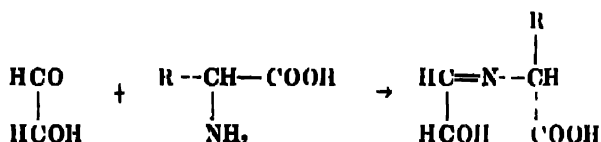
The reaction may take place by direct combination in aqueous or alcoholic solution, but usually it is difficult to isolate the reaction products. Alanine (CH_3—CH_2NH_2—$COOH$) and the ethyl ester of glycine (NH_2—CH_2—$COOC_2H_5$) condense with glucose to give the corresponding N glucosides.[198] Because of the similar conditions of this reaction to those occurring during the dehydration of foods, these syntheses have particular interest.

Cysteine reacts particularly readily with reducing sugars probably because a secondary thiazoline ring is formed.[199]

[196] B. Helferich, W. Richter and S. Grünler, *Ber. Verhandl. sächs. Akad. Wiss. Leipzig, Math.-phys. Klasse,* **89,** 385 (1938).

[197] L. C Maillard, *Ann. chim.,* [11]**5,** 258 (1916), [11] 7, 113 (1917).

[198] J. C. Irvine and A. Hynd, *J. Chem. Soc.,* **99,** 161 (1911); H von Euler and K Zeile, *Ann.,* **487,** 163 (1931).

[199] M. P. Schubert, *J. Biol. Chem.,* **130,** 601 (1939); G. Ågren, *Enzymologia,* **9,** 321 (1941).

$$\text{HSCH}_2\text{—CH—COOH} + \text{glucose} \rightarrow \text{S} \left\langle \begin{array}{c} \text{CH}_2\text{—CH—COOH} \\ | \\ \\ \text{C}\text{==N}^+\text{H} \end{array} \right.$$
$$\underset{\text{NH}_2}{|}$$
$$\underset{\text{HCOH}}{|}$$
$$\underset{\text{HCOH}}{|}$$
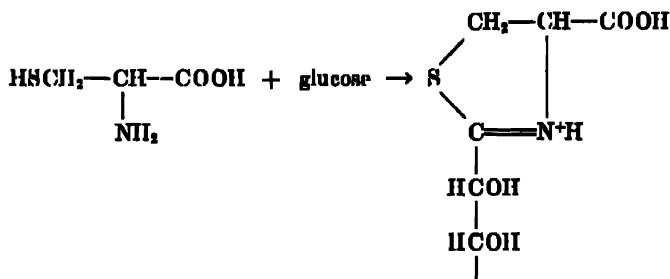
The main evidence for the thiazoline structure is the negative test for —SH groups given with the sodium nitroprusside reagent.

More certain results are obtained by the interaction of the esters or amides of amino acids and tetraacetylglucosyl bromide.[200] The reaction of

$$\left[\begin{array}{c} \text{HCBr} \\ | \\ \text{HCOAc} \end{array}\right] \text{O} + \text{CH}_3\text{NH—CH}_2\text{—CONH}_2 \rightarrow \left[\begin{array}{c} \text{HC—N—CH}_2\text{—CONH}_2 \\ | \quad | \\ \text{CH}_3 \quad \text{O} \\ \text{HCOAc} \end{array}\right] \quad (II)$$
$$\text{(I)}$$
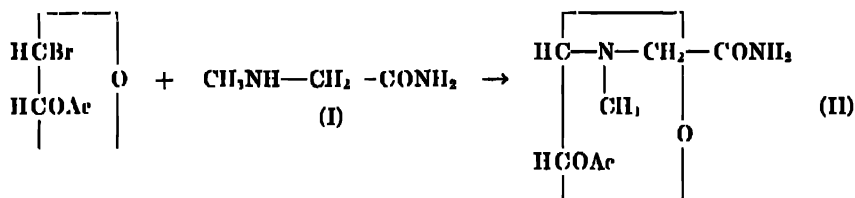
sarcosine amide (I) with tetraacetylglucosyl bromide is illustrated. The tetraacetate (II) yields sarcosine amide N-glucoside upon deacetylation. The glycylglycine N-glucoside and other similar compounds have been made by this method.[201]

Some function of certain amino acids other than the amino group also may be utilized for condensations with sugars. Thus, the phenolic group of tyrosine (p-HO—C_6H_4—CH$_2$—CH(NH$_2$)—COOH) condenses with tetraacetylglucosyl bromide to form an O-glucoside if the amino group is suitably blocked (as with a carbobenzoxy group).[202]

By using the carbobenzoxy method for peptide synthesis, acyl sugar derivatives are obtained in which the acyl group is an amino acid radical.[203] Carbobenzoxyglycyl chloride reacts with the sodium salt of 4,6-benzylideneglucose to form 1-carbobenzoxyglycyl-4,6-benzylidene-D-glucofuranose, which on catalytic hydrogenation gives 1-glycylglucose.

The 5,6-anhydrohexoses react (p. 361) with amino acids with the formation of sugars having amino acids substituted on carbon 6. The 6-desoxy-6-(N-alanino)-glucose (III) is prepared[204] from alanine (II) and 1,2-isopropylidene-5,6-anhydroglucose (I).

[200] K. Maurer and B. Schiedt, Z. physiol. Chem., 208, 125 (1932).

[201] H. v. Euler and K. Zeile, Ann., 487, 163 (1931).

[202] R F. Clutton, C. R. Harington and T. H. Mead, Biochem. J., 31, 764 (1937).

[203] M. Bergmann, L. Zervas and J. Overhoff, Z. physiol. Chem., 224, 52 (1934).

[204] B. Helferich and R. Mittag, Ber., 71, 1585 (1938).

$$\text{(I)} \qquad \text{(II)} \qquad \text{(III)}$$
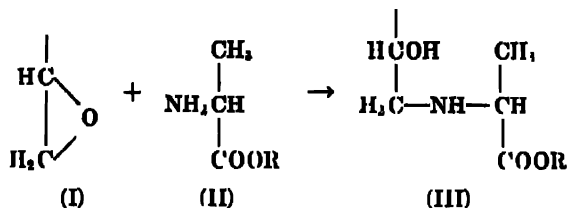
Another procedure for obtaining combinations of sugars and amino acids depends on the acylation of the amino group of aminosugars. The N-glycyl-D-glucosamine or N-alanyl-D-glucosamine is obtained from the action of carbobenzoxyglycyl chloride or carbobenzoxy-*d*-alanyl chloride, respectivey, on tetraacetyl-β-D-glucosamine.[205]

$$(R = p\ CH_3O-C_7H_4-,\ R' = C_6H_5-CH\quad O\quad CO-)$$
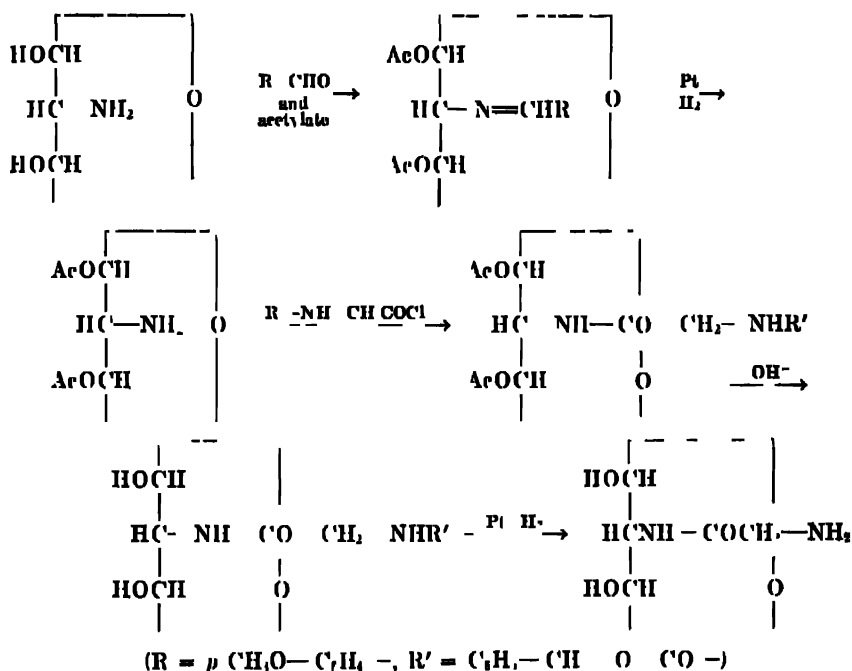
An additional method utilizes the reduction of the tetraacetyl-(N-α-azido-propionyl)-glucosamine and similar derivatives by hydrogen with platinum oxide as catalyst.[206] (See formulas on p. 424.)

The action of some dipeptidase enzymes on such derivatives has been studied by Bergmann and associates[207] and an interesting correlation with

[205] M. Bergmann and L. Zervas, *Ber.*, **65**, 1201 (1932)

[206] A. Bertho and J. Maier, *Ann.*, **495**, 113 (1932), **498**, 50 (1932)

[207] M. Bergmann, L. Zervas, H. Rinke and H. Schleich, *Z. physiol. Chem.*, **224**, 33 (1934)

the enzymic hydrolysis of dipeptides demonstrated. The dipeptides of naturally occurring α-amino acids (those belonging to the *l*-series) and

HCOH
|
HC—NH₂   O $\xrightarrow{N_2\ CH(CH_3)-COCl}$

HCOH
|
HC—NHCO—CH(CH₃)N₃
| ||
HOCH   O

$\xrightarrow[H_2]{Pt}$

HCOH   CH₃
| |
HC—NHCO—CH
| ||
HOCH   O NH
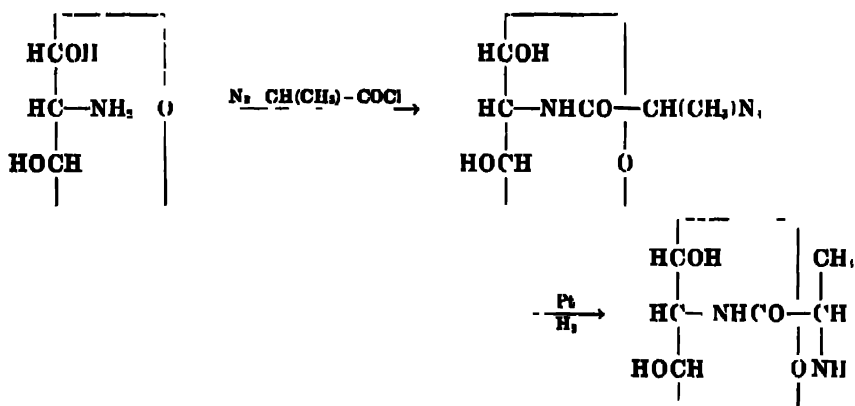
the 2-(glycylamino)-mannonic acid have the same configuration for the asymmetric carbon carrying the substituted amino group; both are hydrolyzed by the dipeptidase. Similar derivatives of 2-desoxy-2-aminogluconic acid (glucosaminic acid) which correspond to dipeptides of the *d*-amino acid series are unaffected by the dipeptidase.

Sugars may be brought into combination with proteins by coupling the proteins with diazonium salts of the glycosides. Goebel, Avery and Heidelberger used this method in their excellent work on the production of synthetic antigens in which the protein is combined with groups of known structure. The diazonium salt is made by the usual procedure of treating an amine with nitrous acid; the amine group in these experiments is in the aglycon group of an aminophenyl glycoside, prepared in turn by reduction of the corresponding nitrophenyl glycoside.[206]

NH₂   N₂⁺Cl⁻   N₂- Protein
| | |
C₆H₄OCH | C₆H₄OCH | C₆H₄OCH |
| O $\xrightarrow{HNO_2}$ | O $\xrightarrow{protein}$ | O
HCOH | HCOH | HCOH |
| | |
                                               (Synthetic
                                                antigen)
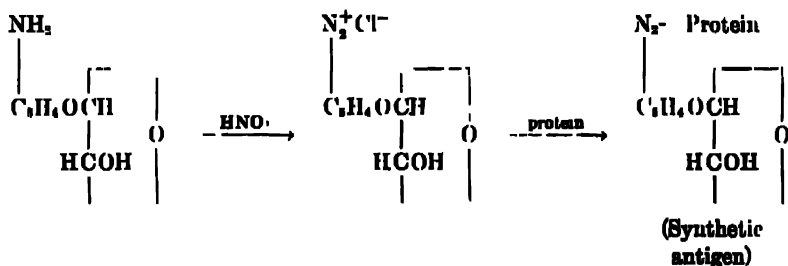
Another process involves coupling the azide formed by the action of nitrous acid on O-β-glucosyl-N-carbobenzoxytyrosine hydrazide with pro-

[206] See O. T. Avery, W. F. Goebel and F. H. Babers, *J. Exptl. Med.*, **55**, 769 (1932). A somewhat similar method is described by B. Woolf, *Proc. Roy. Soc. (London)*, B **130**, 60 (1941).

teins and removing the carbobenzoxy group with the aid of sodium in liquid ammonia.[208]

B. Protein-Carbohydrate Compounds as Synthetic Antigens.[209] Certain substances called antigens induce the formation of antibodies in serum and other body fluids when they are introduced parenterally into animal tissue. The serum which contains the antibodies is known as an antiserum. It reacts specifically with certain antigens as is evidenced by the formation of a precipitate or by other reactions. Synthetic antigens, containing carbohydrates, have been prepared by Avery, Heidelberger, Goebel and associates. These compounds are made as described above. Synthetic antigens of this type were prepared from several proteins and from the glycosides of a number of mono and disaccharides. The antisera formed by the introduction of these antigens into animals were tested for their reaction against the original antigens. It was demonstrated that the principal specificity is related to the carbohydrate rather than to the protein component.[210] For the four antigens

(Protein-I)-β-glucoside (Protein-II)-β-glucoside
(Protein-I)-β-galactoside (Protein-II)-β-galactoside

those formed from different proteins but having the same carbohydrate portion form precipitates with the antisera produced by the use of either as the antigen. Those with the same protein but with different carbohydrate components are serologically different, i.e., neither forms a precipitate with the antiserum produced by the use of the other as the antigen. This behavior is particularly striking since the two proteins alone are serologically different and since the carbohydrates alone do not act as antigens. Many synthetic antigens of this type have been prepared and exhibit similar specificity effects.

Microorganisms frequently form polysaccharides in culture media which, although usually not antigenic, are able to precipitate immune sera prepared against the true antigen, the protein-polysaccharide of the microorganism.[211] The pneumococcus polysaccharides have received the most study and these are specific for the various types (strains) of pneumococci. These microorganisms have capsules which have been shown to consist of the type-specific polysaccharides. From the polysaccharide of the type-III pneumococcus, a synthetic antigen was prepared by diazotization of the p-aminobenzyl ether of the polysaccharide and then coupling with serum

[208] J. Marrack, *Ergeb. Enzymforsch* , 7, 281 (1938)
[210] W F Goebel, O T. Avery and F H. Babers, *J Exptl. Med* , 60, 599 (1934)
[211] M Heidelberger and O T Avery, *J Exptl.Med.,40,301* (1924); W. T J Morgan, *Biochem. J* , 30, 909 (1936)

globulin.[212] This antigen evoked an antiserum exhibiting reactions similar to those of the antiserum produced by type III pneumococcus. The constitution of some of these polysaccharides is discussed later (Chapter XV). They usually contain uronic acids and/or amino sugars. It is then of considerable interest that synthetic antigens, prepared by the above procedure from the p-nitrobenzyl glucuronides, gentiobiuronides and cellobiuronides confer immunity against pneumococci. All of these protein-azobenzyl uronides evoke antisera in rabbits which, when introduced into mice, protect them (passive immunity) against type II pneumococcal infection. Although the cellobiuronide antiserum from rabbits produces a temporary (passive) immunity to type III and VIII pneumococcal infections in mice, the gentiobiuronide antiserum is ineffective. The corresponding antisera prepared from the glycosides of galacturonic acid, cellobiose and gentiobiose fail to protect mice against pneumococcal infection by these types.[213]

[212] W. F. Goebel and O. T. Avery, *J. Exptl. Med.*, **54**, 431 (1931).
[213] W. F. Goebel, *Science*, **91**, 20 (1940); *J. Exptl. Med.*, **72**, 33 (1940)

CHAPTER X

OLIGOSACCHARIDES

Frequently found free or combined in natural products is a group of carbohydrates (of lower molecular weight than the polysaccharides) which on complete acid hydrolysis yield only simple sugars or their derivatives such as the uronic acids and amino sugars. These products, known[1] as oligosaccharides (Greek *oligos*, a few), are composed of monosaccharide residues connected through glycosidic linkages. On the basis of the number of monosaccharide residues per mole, the oligosaccharides are classified as disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, etc. (p. 17). No sharp distinction can be drawn between the oligosaccharides and the polysaccharides, for the structures are similar and only the molecular weights are different. For the present, it is sufficient to limit the term oligosaccharide to the lower members of each polymeric series; in the present discussion the term will be limited to substances with less than ten monosaccharide residues in the molecule since this limit permits the inclusion of all the well-defined substances. In general, the polysaccharides have a much greater degree of polymerization, in some cases several thousand.

The oligosaccharides also may be considered as glycosides, for the linkages connecting the monosaccharide residues are oxygen bridges between the hemiacetal hydroxyl of the anomeric carbon of one residue and an alcoholic hydroxyl of another residue. It is sometimes convenient to distinguish glycosides having an alcoholic or phenolic aglycon group from those having a sugar or oligosaccharide radical as the nonglycosidic portion of the molecule by designating them as "heterosides" and "holosides," respectively.

Since the oligosaccharides may be considered as either glycosides or substituted sugars, several types of names are possible. Thus, lactose may be considered to be either a β-galactoside or a 4-substituted glucose. It could be named 4-glucose β-galactoside or 4-(β-galactosyl)-glucose.[2]

According to the presence or absence of reducing groups in the molecule,

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[1] B. Helferich, E. Bohn and S. Winkler, *Ber.*, *63*, 989 (1930).

[2] The terms "glycoside" and "glycosyl" have been used as synonyms in much of the older literature. In the present work, glycosyl will be used and will refer to the radical obtained by removal of the anomeric hydroxyl from a reducing sugar.

In the present text, the number referring to the position of attachment of the glycosyl radical to the reducing residue is placed before the residue to which it refers, *e.g.*, 4-glucose β-galactoside. This is contrary to usage such as glucose 6-benzoate, but it is used here because of the confusion which would arise from the presence of other substituent groups.

<div style="text-align:center">

TABLE I

Classification of Oligosaccharides

</div>

I Nonreducing oligosaccharides

  A Those yielding a single monosaccharide type on hydrolysis

    Trehalose (a disaccharide, see p. 452).

    Scorodose[1]: A tetrafructoside from *Allium* bulbs (onion and garlic)

    Schardinger dextrins (see under Starch)

  B. Those yielding two or more monosaccharide types on hydrolysis

    Disaccharides:

      Sucrose (see p. 446).

    Trisaccharides:

      Melezitose (see p. 455).

      Raffinose (see p. 456).

      Gentianose (see p 455).

      Labiose[4], from *Eremostachys labiosa*. The structure is uncertain, but the sugar is hydrolyzed to two moles of fructose and one of galactose

    Tetrasaccharides:

      Stachyose (see p. 458)

    Pentasaccharides.

      Verbascose (galactosylstachyose) from *Verbascum thapsus* a mullein (see under Stachyose)

II Reducing oligosaccharides

  A Disaccharides

    1 With 1,4' linkages:

      Maltose (see p. 443)

      Cellobiose (see p. 438)

      Lactose (see p. 440)

      4-D-Xylose D-glucuronide[5] from mucilage of *Kadsura japonica* Don

      D-Glucuronic acid D-glucuronide,[6] the sugar component of the glycoside glycyrrhizin, the sweet principle of *Glycyrrhiza glabra* (licorice) The evidence for a 1,4' connection and for the identification of the uronic acid is not complete

      Scillabiose[7], 4-L-rhamnose D-glucoside, obtained by hydrolysis of scillaren-A, a glycoside of the squill *Scilla maritima* L.

    2. Primary hydroxyl involved in disaccharide formation (1,6' or 1,5' linkages) With the exception of melibiose and isomaltose, these substances are frequently found as the sugar constituents of natural glycosides

      Gentiobiose (see p. 439)

      Melibiose (see p. 445)

      Isomaltose (see p. 444).

      Vicianose,[8,9,10] 6 D-glucose α-L-arabinoside, the sugar component of the glycoside vicianin (related to amygdalin) found in the vetch *Vicia angustifolia*. This compound is described in the literature as both an α-L- and β L-arabinoside. Using the accepted nomenclature of Hudson, it should be termed the α L-isomer (see under Arabinose).

      Primeverose,[10,11,12] 6-D-glucose β-D-xyloside, found in many glycosides, *e.g.*, the alizarin primeveroside of madder (see Ruberythric acid).

      Rutinose[13], 6-D-glucose β-L-rhamnoside. the sugar component of several glycosides including rutin from rue (*Ruta graveolens*).

      Robinobiose[13], 6-D-galactose β-L-rhamnoside, obtained by partial hydrolysis of the glycoside robinin from locust flowers (*Robinia pseudoacacia*).

TABLE I—*Concluded*

II. Reducing oligosaccharides—*Continued*

  3 With 1,3' linkages·
    Turanose (see p. 453)
    Laminaribiose,[14] probably 3-D-glucose β-glucoside Obtained by partial hydrolysis of the polysaccharide laminarin occurring in brown algae (*Laminaria*).
    "Amylolyose,"[15] 3-D-glucose β-glucoside-?, obtained by enzyme hydrolysis of starch
    3-L Arabinose D-galactoside,[16] from arabic acid (gum arabic).
  4 With 1,1' linkages:
    1-D-Fructose β-D-glucoside[17] (synthetic).
  5. With 1,2' linkages:
    Sophorose,[18] 2-D-glucose β-D-glucoside, from sophoraflavonoloside. A similar compound has been synthesized.
    2-L-Rhamnose D-galacturonide,[19] a product of the partial hydrolysis of flaxseed mucilage
  6. With 1,5' linkages.
    5-D-Arabinose D-glucoside[20] (synthetic, amorphous)
B. Reducing trisaccharides
    Manninotriose, 4 D-glucose 6 D-galactosyl-galactoside, from ash manna of *Fraxinus ornus* (see under Stachyose)
    Rhamninose[21], D-galactose L rhamnosyl-L-rhamnoside, prepared from the glycoside xanthorhamnin of the Persian berry *(Rhamnus infectoria)* by the action of enzymes found in the same plant
    Robinose [22] L rhamnose L rhamnosyl D-galactoside derived from the glycoside robinin by enzymic hydrolysis

These references usually are to only the most recent work on the subject. Earlier references may be located by consulting either the references cited or Beilstein

  [3] Y. Kihara, *J. Agr Chem Soc Japan*, 15, 348 (1939), *Chem. Abst.*, 34, 385 (1940)
  [4] S M Strepkov, *J Gen Chem (U S S R)* 9 1189 (1939), *Chem. Abst.*, 34, 2798 (1940)
  [5] K Nishida and H Hashima, *J Agr Chem. Soc Japan*. 13, 660 (1937); *Chem Abst.*, 32, 4112 (1938)
  [6] W. Voss and J Pfirschke, *Ber.*, 70, 132 (1937)
  [7] G. Zemplén, *Chem. Abst.*, 33, 4202 (1939)
  [8] G Bertrand and G. Weisweiller, *Compt. rend.*, 150, 180 (1910)
  [9] B. Helferich and H. Bredereck. *Ann.*, 465, 166 (1928)
  [10] C. M. McCloskey and G. H. Coleman, *J. Am. Chem. Soc.*, 65, 1778 (1943)
  [11] G. Zemplén and R. Bognár, *Ber.*, 72, 17, 1160 (1939)
  [12] A. Goris, M Mascré and Ch. Vischinniac, *Bull sci pharmacol.*, 19, 587 648 (1912).
  [13] G. Zemplén and A. Gerecs, *Ber.*, 71, 774, 2520 (1938); 68, 2054 (1935).
  [14] V. C Barry, *Sci. Proc. Roy. Dublin Soc.*, 22, 423 (1941).
  [15] Y Nakamura, *J. Agr. Chem. Soc Japan*, 17, 603 (1941); *Chem. Abst.*, 36, 5049 (1942).
  [16] F. Smith, *J. Chem. Soc.*, 744 (1939).
  [17] P. Brigl and O. Widmaier, *Ber.*, 69, 1219 (1936); E. Pacsu, E. J Wilson, Jr., and L. Graf, *J. Am. Chem. Soc.*, 61, 2675 (1939).
  [18] J. Rabaté, *Bull. soc. chim.*, [5] 7, 565 (1940); A. M. Gakhokidze, *J. Gen. Chem. (U.S.S.R.)*, 11, 117 (1941); *Chem. Abst.*, 35, 5467 (1941).
  [19] R. S. Tipson, C. C. Christman and P. A. Levene, *J. Biol. Chem.*, 128, 609 (1939).
  [20] N. S. MacDonald and W. L. Evans, *J. Am. Chem. Soc.*, 64, 2731 (1942).
  [21] C. and G. Tanret, *Bull. soc. chim.*, [3] 21, 1065 (1899).
  [22] C. Charaux, *Bull. soc. chim. biol.*, 8, 915 (1926).

the unsubstituted oligosaccharides are conveniently classified as reducing and nonreducing. This property is important, for it provides a test for the existence of a monosaccharide residue with unsubstituted hemiacetal hydroxyls. When such unsubstituted groups are present, the sugar reduces alkaline copper-salt solutions, mutarotates, and forms glycosides and osazones similarly to the monosaccharides. In the absence of a reducing group, none of these reactions are exhibited, and only the reactions of hydroxyl groups are shown by the unhydrolyzed sugar.

1. Individual Oligosaccharides and Their Classification

In Table I, which includes most naturally occurring oligosaccharides of known structure and a few synthetic sugars, the primary classification is made on the basis of the presence or absence of reducing groups. For the reducing disaccharides, a further subdivision based on the position of the glycosidic linkage is made. Since the more important oligosaccharides are described in detail later in this chapter, their structure and origin are not given in Table I. Literature references and some structural data are given for the compounds which in subsequent discussion are not considered in detail. Aldobiuronic acids are discussed on p 306.

2. Synthesis of Oligosaccharides

A number of methods are available for the synthesis of oligosaccharides and in particular of the disaccharides. Many of the methods useful for the interconversion of the simple monosaccharides may also be applied to the disaccharides. Other methods involve the condensation of two moles of monosaccharides or derivatives. To contribute to the knowledge of the structure of a particular disaccharide, the synthetic method must be such that the condensation reaction takes place between known positions as otherwise several structural isomers are possible. The restriction of the reaction to predetermined positions is accomplished by blocking all the hydroxyl groups by easily removable groups such as acetyls except those between which the condensation is to occur. Additional complications are introduced when asymmetric carbons are involved since it must be known whether Walden inversions take place. Probably the most useful reaction is the Koenigs-Knorr synthesis which depends on the reaction of the acetylglycosyl halides with the unsubstituted hydroxyls of a second monosaccharide molecule.

A. From Naturally Occurring Oligosaccharides. These methods are usually applicable only to the reducing oligosaccharides, *i.e.*, those having an unsubstituted hemiacetal grouping, since most isomerizations involve the reducing group.

Alkaline Rearrangement. The reducing disaccharides isomerize in the

presence of alkali to give a mixture of three sugars which consist of the two 2-epimeric aldoses and the corresponding ketose. This action of dilute alkali is the same as that on the monosaccharides (Chapter II). The method is particularly important for obtaining ketose disaccharides but has, been applied in only a few cases. Lactose yields[24] lactulose (4-fructose β-galactoside) which is exceptionally interesting because the crystalline sugar is apparently a furanose form. The pyridine rearrangement either of the oligosaccharides or of their carboxylic acids has never been utilized for this purpose.

Glycal Synthesis. This method depends upon the preparation of a glycal (which has a double bond between carbons 1 and 2 of the reducing residue of the molecule) and the oxidation of the glycal with perbenzoic acid to give two 2-epimeric aldoses as described previously for the monosaccharides (Chapter II). The epimers of lactose, maltose and cellobiose have been obtained by this method.[21]

Degradation Reactions. By use of the several methods described for shortening the carbon chain of the monosaccharides (Chapter III), the reducing carbon of the disaccharides also may be removed. Calcium lactobionate is oxidized by hydrogen peroxide with ferric salts as catalyst to 3-D-arabinose β-D-galactoside,[25] and acetylated gentiobionic acid nitrile gives[26] 5-D-arabinose D-glucoside by the action of sodium methylate (Wohl-Zemplén method) This sugar is of particular interest as it cannot form a pyranose ring.

Walden Inversions Several reactions, not of general application, are of importance for obtaining special disaccharides. Cellobiose octaacetate, when subjected to the procedure which usually produces the acetylglycosyl fluorides, gives the expected product and the corresponding derivative of the 2-epimeric disaccharide 4-mannose β-glucoside.[26] In this instance, the action of anhydrous hydrogen fluoride inverts the configuration of the second carbon and also replaces the acetoxy of carbon 1 by a fluorine atom. In a somewhat similar manner, lactose and cellobiose octaacetate under chlorinating conditions (aluminum chloride and phosphorus pentachloride in chloroform solution) yield, in addition to the expected heptaacetyl-glycosyl chloride, isomeric compounds produced by Walden inversions at carbons 2 and 3 of the reducing part of the molecules. The new disaccharides are neolactose (4-D-altrose β-D-galactoside) from lactose and celtrobiose (4-D-altrose β-D-glucoside) from cellobiose.[27] These disaccharides

[23] E. Montgomery and C. S. Hudson, *J. Am. Chem. Soc.*, **52**, 2101 (1930).

[21] M. Bergmann and H Schotte, *Ber.*, **54**, 1564 (1921); W N. Haworth, E. L. Hirst, et al , *J. Chem Soc* , 2636, 2644 (1930)

[26] G. Zemplén, *Ber* , **60**, 1309 (1927); O. Ruff and G Ollendorff, *ibid* , **33**, 1798 (1900).

[24] D. H Brauns, *J. Am. Chem Soc* , **48**, 2776 (1926)

[27] A. Kunz and C S Hudson, *J. Am Chem. Soc.*, **48**, 1978, 2135 (1926); N. K Richtmyer and C. S Hudson, *ibid* , **57**, 1716 (1935); **58**, 2534 (1936).

are of particular importance in providing new sources for D-altrose, a sugar difficult to obtain by older methods.

Octaacetyl
lactose

Heptaacetylneolactosyl
chloride

(Gal = galactosyl group)

Disaccharide Methyloses (6,6'-Didesoses). The tosyl groups of 6,6'-ditosylcellobiose are replaceable with iodine by the action of sodium iodide in acetone solution. The resulting 6,6'-diiodocellobiose may be reduced by catalytic hydrogenation to 6,6'-didesoxycellobiose.[23] A similar compound with one 6-desoxy and one primary hydroxyl group is obtained by the addition of tetraacetylglucosyl bromide to the anhydro ring of 5,6-anhydroglucose (see below).

B. Condensation of Two Monosaccharide Molecules. *Koenigs-Knorr Reaction.* This reaction, described elsewhere in detail for the preparation of the glycosides, is probably the most widely applicable and important of the methods. An acetylglycosyl halide, usually the bromide, reacts with the unsubstituted hydroxyl of a second monosaccharide molecule in the presence of silver carbonate or silver oxide with the formation of an oxygen glycosidic linkage between the two molecules. In most instances the glycosidic carbon has the β-configuration as is demonstrated by the formation of β-glycosides when an alcohol rather than a second monosaccharide molecule is employed in the reaction. The process involves a Walden inversion and usually proceeds smoothly in high yields when the halogen and the acetyl group on carbons 1 and 2 respectively have a *cis* relation. If they are *trans*, however, orthoesters may be formed (p. 163). Disaccharides with glucose, galactose, arabinose and xylose constituting the glycosidic portion of the molecule are easily obtained in this manner. When it is desired to synthesize an α-glucosidic linkage, the use of mercuric acetate or pyridine as the catalyst rather than silver salts often gives the desired results. Melibiose (6-glucose α-galactoside) is synthesized by the action of tetraacetylgalactosyl bromide on 1,2,3,4-tetraacetylglucose in the presence of quinoline. Condensation of triacetylxylosyl bromide and a glucose derivative having an unsubstituted hydroxyl group at carbon 6 may take place

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[23] J. Compton, *J Am. Chem. Soc.*, *60*, 1203 (1938).

in the presence of mercuric acetate exclusively with inversion of configuration and the formation of a primeverose (6-glucose β-D-xyloside) derivative.[29] If the amount of catalyst employed is small, an additional reaction without inversion takes place with the simultaneous formation of iso-primeverose (6-glucose α-D-xyloside) which has an α-glycosidic linkage. Disaccharides with linkages through primary alcoholic groups (gentiobiose type) are easily obtained by these methods since the necessary monosaccharide derivatives with free primary hydroxyl groups and with the other groups blocked are readily obtained from the corresponding trityl derivatives.

Tetraacetylglucosyl   1,2,3,4-Tetraacetyl   Octaacetylgentiobiose
    bromide   glucose
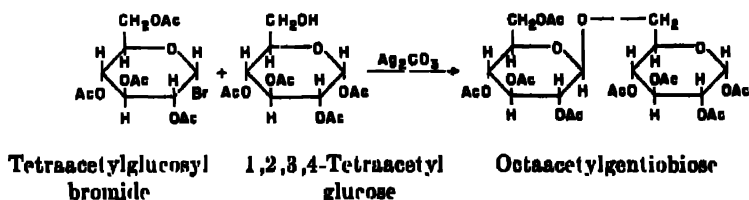
The method may be applied to the preparation of trisaccharides by utilizing a 1-halogeno acetylated disaccharide in place of tetraacetylglucosyl bromide. The 6-glucose and 6-mannose β-cellobiosides and the 6-glucose β-maltoside are prepared in this manner.[30] Dihydroxyacetone can be condensed with acetylglycosyl halides to form simple types of disaccharides.[31] The dihydroxyacetone D-riboside is particularly interesting in that the acetylated compound has an orthoester structure.

It is more difficult to obtain sugar derivatives with single free hydroxyls other than glycosidic or primary. However, valuable derivatives of this type are the isopropylidene sugars and the isopropylidene-anhydrosugars. By condensation of 2,3-isopropylidene-1,6-anhydromannopyranose with tetraacetylgalactosyl bromide, subsequent hydrolysis of the isopropylidene and acetyl groups, and cleavage of the anhydro ring with acids, 4-mannose β-D-galactoside (epilactose) is obtained. The epilactose is then carried through the glycal synthesis to produce lactose. 4-Mannose β-D-glucoside (epicellobiose) and cellobiose are prepared by the same procedure from tetraacetylglucosyl bromide and the 2,3-isopropylidene-1,6-anhydromannopyranose. More details of these methods are given under Cellobiose, Gentiobiose, Melibiose and Lactose later in this chapter. When 1,2-isopropylidene-5,6-anhydro-glucofuranose (I) reacts with tetraacetylglucosyl bromide condensation takes place with the simultaneous opening of the

[29] G. Zemplén and R. Bognár, Ber., 72, 1160 (1039).

[30] B. Helferich and W. Schäfer, Ann., 450, 229 (1926); S. H. Nichols, Jr., W. L. Evans and H. D. McDowell, J. Am. Chem. Soc., 62, 1754 (1940).

[31] C. W. Klingensmith and W. L. Evans, J. Am. Chem. Soc., 61, 3012 (1939).

anhydro ring, addition of the glucosyl group to the oxygen attached to carbon 5 and addition of bromine to carbon 6. Catalytic reduction replaces the bromine by a hydrogen atom, and there is obtained an unusual disaccharide methylose derivative, 5-(6-desoxyglucose) β-D-glucoside (II).[32]
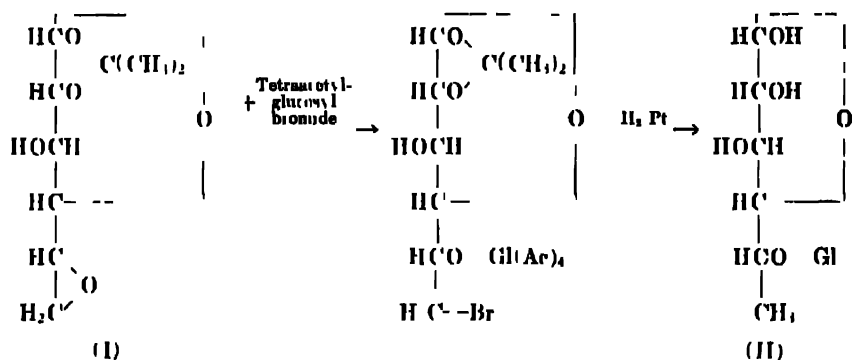
$Gl(Ac)_4$ = Tetraacetylglucosyl group,

Gl = Glucosyl group

Thermal Condensations. Two moles of sugar condense when heated *in vacuo.* Maltose is said to be produced in this manner from β-glucose and α-glucose and from β-glucose and β-glucosan. The use of zinc chloride improves the yields. The maltose is separated as the acetate in yields of 5 to 10 per cent.[33] Lactose may have been obtained in this manner from β-glucose and either β-galactose or β-galactosan.[14] The method, however, does not provide any information as to the structures of the sugars, and it would be very desirable that this work be repeated.

Direct Catalyzed Condensation of Monosaccharides in Solution. In the presence of acids and water, the disaccharides are in equilibrium with the products of hydrolysis although the rate of attainment of equilibrium is very slow at room temperature. An excess of water favors the existence of the monosaccharides in the equilibrium mixture, whereas a high concentration of the sugar is favorable to the existence of disaccharides and oligosaccharides. A 25 per cent solution of glucose in concentrated hydrochloric acid gives, after 15 hours at room temperature, a mixture from which a disaccharide osazone (isomaltose) may be isolated.[35] As might be

$$2C_6H_{12}O_6 \xrightleftharpoons[\text{}]{H^+} C_{12}H_{22}O_{11} + H_2O$$
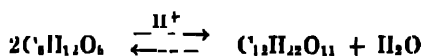
[32] K. Freudenberg, H. Eich, C. Knoevenagel and W. Westphal, *Ber.*, **73**, 441 (1940).
[33] A. Pictet and H. Vogel, *Helv. Chim. Acta*, *10*, 588 (1927)
[14] A. Pictet and H. Vogel *Helv. Chim. Acta*, *11* 200 (1928)
[35] See: E. Fischer, *Ber.*, *23*, 3687 (1890)

expected from the many positions available for formation of a disaccharide linkage and because each position allows for at least one pair of α-β-isomers, the products formed are probably complex mixtures. However, the available evidence indicates a preference for condensation between the hemiacetal (anomeric) hydroxyl and the primary hydroxyl groups. This type of reaction is very important in industrial processes involving the hydrolysis of polysaccharides such as in the preparation of D-glucose from starch. The specificity of the reaction may be greatly increased by the use of enzymes rather than acids as the catalysts. According to the enzyme employed, α- or β-glycosidic linkages may be synthesized at will, and even the position of condensation in the nonglycosyl sugar may be varied somewhat. Maltose and other α-glucosides are formed by the action of yeast α-glucosidase on concentrated glucose solutions.[36] Similarly, gentiobiose and cellobiose are formed from glucose solutions through the catalytic action of β-glucosidases, and the relative proportions of the two isomers is affected by the concentration.[37] Gentiobiose is also formed by the action of dried yeasts on glucose solutions.[38]

In many cases the synthesis of oligosaccharides under natural conditions probably takes place as a result of phosphorylase action. Such an action has been demonstrated by the *in vitro* synthesis of sucrose from glucose 1-phosphate and fructose in the presence of enzymes from bacteria such as *Pseudomonas saccharophila* (see under Sucrose).

Elimination of Water from Two Monosaccharide Units by Use of Dehydrating Agents. Acetylated sugars with free hydroxyl groups may be condensed with the elimination of a mole of water. Two moles of 2,3,4,6-tetraacetylglucose combine[39] under the influence of phosphorus pentoxide in an inert solvent such as benzene with the formation of the octaacetate of 1-β-glucose β-glucoside: β,β-trehalose (see under Sucrose).

3. Determination of Structure

The methylation method has been the principal means for the determination of the structure of oligosaccharides. For the disaccharides the method is applied by methylating the sugars or better the aldobionic acids and then hydrolyzing them to the constituent monosaccharides. The position of the disaccharide linkage is shown by the position of the free hydroxyl groups in the resulting monosaccharides. The use of the aldobionic acids is

[36] A. C. Hill, *J. Chem. Soc.*, **83**, 589 (1903).

[37] E. Bourquelot, H. Hérissey and J. Coirre, *Compt. rend.*, **157**, 732 (1913); I Vintilescu, C. N. Ionescu and A. Kizyk, *Bull. soc. chim. Roumania*, **17**, 283 (1935)

[38] H. Pringsheim, J Bondi and J. Leibowitz, *Ber.*, **59**, 1083 (1926).

[39] E. Fischer and K Delbrück, *Ber* . **42**, 2776 (1909); F. Klages and R Niemann, *Ann.*, **529**, 185 (1937)

desirable because the sugars are methylated in their ring forms; when the methylated acid is hydrolyzed, a free hydroxyl group appears only in the position to which the disaccharide bridge was connected. Methyl hepta-methylmaltoside is hydrolyzed to 2,3,4,6-tetramethylglucose and 2,3,6-trimethylglucose whereas methylated maltobionic acid gives 2,3,4,6-tetramethylglucose and 2,3,5,6-tetramethylgluconic acid (see under Maltose). The position of the free hydroxyl of the tetramethylgluconic acid shows that the disaccharide must be connected at the 4-position of this residue.

The methylation method works well for disaccharides when the necessary reference compounds (methylated monosaccharides) are known. Additional methods are necessary for the application to oligosaccharides higher than disaccharides (see under Raffinose). The action of enzymes may be valuable for this purpose and for providing information concerning the configuration of the disaccharide (glycosidic) linkage.

Periodic acid oxidation has had only limited application in structural studies of oligosaccharides When the method was applied to sucrose, the results corroborated those by the methylation method (see under Sucrose) According to Ahlborg,[40] it is possible to distinguish between 1,4' and 1,6' linkages in disaccharides by oxidation with periodic acid and with lead tetraacetate. When oxidized by periodic acid in acid solution, disaccharides with 1,4' linkages and with two primary alcoholic groups yield two moles of formaldehyde (isolated by precipitation with Dimedon, 1,1-dimethyl-cyclohexanedione-3,5), whereas those having 1,6' linkages and only one primary alcoholic group yield no formaldehyde. Free iodine is liberated stoichiometrically from oligosaccharides having 1,4' glycosidic bonds and provides a measure of the number of such bonds present. The iodine may arise from the reaction of periodic acid with reductone which is formed from the reducing portion of the disaccharide
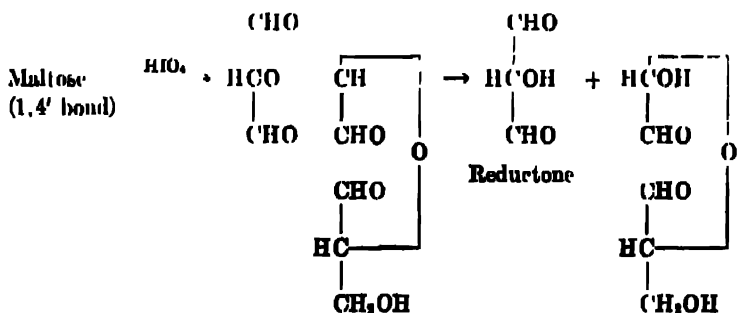
[40] K. Ahlborg, *Svensk Kem. Tid.*, **54**, 205 (1942); *Chem. Abst.*, **38**, 4254 (1944)
R. Jeanloz, *Helv. Chim. Acta*, **27**, 1501 (1944).

It is probable that the glycosidic union is not cleaved during the action of periodic acid on 1,6′ disaccharides and that the oxidation proceeds as follows:

$$
\text{Gentiobiose} \xrightarrow{\text{HIO}_4} \text{H}_2\text{CO} - \text{CH}
$$

Gentiobiose
(1,6′ bond)

CHO
|
H₂CO - -CH
|
CHO   O
|
CHO
|
HC
|
CH₂OH
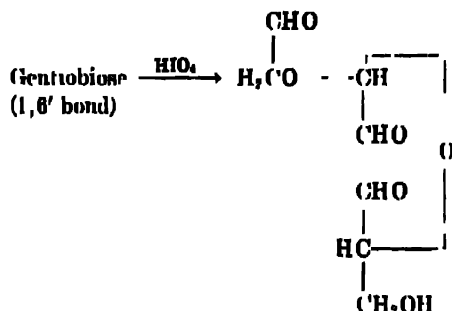
TABLE II

Ease of Acid Hydrolysis of Some Oligosaccharides

Oligosaccharide	$K/a_H^+ \times 10^5$ (sec.$^{-1}$)	Activation energy (cal /g. mole)
1 α-Glucose α-glucoside (Trehalose)	0.861	40,180
6-Glucose β glucoside (Gentiobiose)	1.24	33,390
4-Glucose β-glucoside (Cellobiose)	5.80	30,710
3-Fructose α glucoside (Turanose)	11.0	32,450
4-Glucose α glucoside (Maltose)	16.8	30,970
4-Glucose β-galactoside (Lactose)	16.6	26,900
6-Glucose α-galactoside (Melibiose)	15.5	38,590
1-α-Glucose β-fructofuranoside (Sucrose)	14,600	25,830
Raffinose	11,200ᵃ	25,340
Melezitose	48,300ᵃ	25,600

ᵃ These figures presumably represent the hydrolysis of the sucrose linkage in these trisaccharides

4. Ease of Acid Hydrolysis

As shown in the above table,[41] there is considerable difference in the ease of hydrolysis of oligosaccharides; sucrose, with its fructofuranose ring, is particularly labile. The ease of hydrolysis of the sucrose linkage in comparison with that of the glucopyranosides makes it possible to hydrolyze preferentially the sucrose linkage in trisaccharides with the formation of a resistant disaccharide. Thus, turanose, a disaccharide, is prepared by the partial hydrolysis of the parent trisaccharide melezitose.

41 E. A. Moelwyn Hughes, *Trans. Faraday Soc.*, **25**, 503 (1929).

5. Preparation, Properties and Structures of Some Natural
Oligosaccharides[42]

A. Disaccharides
Cellobiose
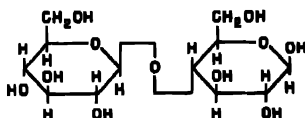
Synonyms. 4-D-Glucose β-D-glucopyranoside, 4-(β-D-glucopyranosyl)-D-glucose.

Properties. β-Isomer; m.p., 225°C.; $[\alpha]_D^{20} = + 14.2 \rightarrow + 34.6$ (H₂O; c, 8).

Identification. Phenylosazone, octaacetate.

Occurrence. The sugar is not known to exist in the free state in products of biological origin but is the basic repeating unit of cellulose and lichenin.

Preparation.[42,43] Cellulose in the form of cotton or filter paper is simultaneously acetylated and acetolyzed by the action of acetic anhydride and sulfuric acid at low temperatures. Cellobiose octaacetate crystallizes from the reaction mixture and after separation is recrystallized from alcohol. The acetyl groups are removed by any of several methods, preferably with barium methylate in methyl alcoholic solution.

Structure. See discussion under Maltose.

Synthesis. By the reaction of 1,6-anhydroglucose (levoglucosan) with tetraacetylglucosyl bromide and the subsequent hydrolysis of the anhydro ring with sulfuric acid, Freudenberg and Nagai[44] were able to synthesize cellobiose in small yield. The method provides evidence for the beta configuration of the glucosidic linkage, for β-glucosides are formed when alcohols react with tetraacetylglucosyl bromide under the same conditions (Koenigs-Knorr synthesis). The exact position of the glucosidic linkage is not defined, however, as several unsubstituted hydroxyl groups are present in the 1,6-anhydroglucose. A structurally definitive synthesis by the reaction of 2,3-isopropylidene-1,6-anhydromannopyranose (which contains only one free hydroxyl, at carbon 4) and tetraacetylglucosyl bromide has

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[42] For more detailed information concerning the occurrence and preparation of many of the individual oligosaccharides, the following references are particularly recommended:

F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars" Circular C440 of the National Bureau of Standards; U. S. Government Printing Office, Washington, D. C. (1942).

"Beilsteins Handbuch der organischen Chemie," Vol. *31*; J. Springer, Berlin (1938)

[43] A. P. N. Franchimont *Ber.*, *12*, 1941 (1879); G. Braun, *Organic Syntheses*, *17*, 34, 36 (1937).

[44] K. Freudenberg and W. Nagai, *Ber.*, *66*, 27 (1933)

been described.[46] After rupture of the anhydro ring, a derivative of the 2-epimer of cellobiose is obtained (epicellobiose) which is carried to cellobiose through the intermediary cellobial (epicellobial). The method is similar to that used for the synthesis of lactose. (See under Lactose.)

Metabolism. Absorption of the disaccharide in the gut of the rat takes place at about 6.8 per cent of the rate of absorption of glucose.[46] Normal rats form glycogen in the liver and muscle in equivalent amounts from cellobiose and glucose. These two sugars possess the same ability to lower an exogenous ketonuria.

Gentiobiose
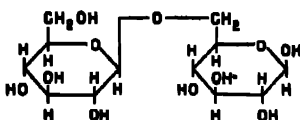
Synonyms. 6-Glucose β-D-glucopyranoside, 6-(β-D-glucopyranosyl)-D-glucose, amygdalose.

Properties. α-isomer; crystallizes associated with two moles of methyl alcohol; m.p., 85–86°C., $[\alpha]_D^{20} = + 21.4 \rightarrow + 8.7$ (H_2O; $c,5$). β-Isomer (solvent free); m.p., 190–195°C. Not fermented by top yeasts.

Identification. Phenylosazone, octaacetate.

Occurrence. The disaccharide is the sugar constituent of a number of glycosides of which the most important are amygdalin and crocin. The two glucose units of the trisaccharide gentianose, which is found free in the roots of plants of the *Gentian* species, are connected together in the same manner as gentiobiose. Gentiobiose is found in materials obtained by the action of acids and certain enzymes on D-glucose and on polymers such as starch and cellulose. In such materials it is probably a reversion product (see under Isomaltose).

Preparation.[42][47] The sugar is obtained by partial acid or enzymic hydrolysis of gentianose and removal of the fructose by yeast fermentation. It is usually separated as the octaacetate. The hydrogenation of heptaacetyl-amygdalin[48] and the isolation of the octaacetate of gentiobiose from the acetylated mother liquors ("hydrol") of the preparation of D-glucose from starch have been suggested for the preparation of the disaccharide. The best method, however, is probably the synthetic method of Helferich as

[45] W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am Chem. Soc.*, **64**, 1289 (1942).

[46] C. E. Vaniman and H. J Deuel, Jr., *J. Biol. Chem.*, **152**, 565 (1944).

[47] C. S. Hudson and J. Johnson, *J. Am. Chem. Soc.*, **39**, 1272 (1917); E. Bourquelot and H. Hérissey, *Compt. rend.*, **135** 290 (1902).

[48] M. Bergmann and W. Freudenberg, *Ber.*, **62**, 2783 (1929).

modified by Reynolds and Evans and described below. Enzymic synthesis by the action of almond emulsin on glucose is also recommended.[49]

Synthesis.[50] By the condensation of 1,2,3,4-tetraacetyl-β-D-glucose with tetraacetylglucosyl bromide, a linkage is established between carbon 6 of the one molecule and carbon 1 of the second molecule, and octaacetyl-gentiobiose is formed.

Structure.[51] Methylation of gentiobiose followed by acid hydrolysis gives the well-known 2,3,4,6-tetramethylglucose and a trimethylglucose yielding a crystalline methyl trimethylglucoside. The latter substance is identical with that formed by the methylation and detritylation of methyl 6-trityl-glucopyranoside and must be methyl 2,3,4-trimethylglucoside. This evidence makes it certain that the disaccharide linkage of gentiobiose connects carbon 1 of one glucose unit and carbon 6 of the second glucose unit. It is confirmed by the above synthesis under conditions such that the linkage can be formed only between these two carbons.

The β-configuration for the glucosidic linkage is shown by the hydrolysis of gentiobiose by the β-glucosidase of almond emulsin and by its synthesis under conditions favorable to the formation of β-glucosides (Koenigs-Knorr synthesis).

Lactose[52]
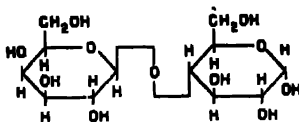
Synonyms 1-D-Glucose β-D-galactopyranoside, 4-(β-D-galactopyranosyl)-D-glucose, milk sugar.

Properties Monohydrate of α-isomer · m.p., 202°C; $[\alpha]_D^{20}$ − + 85.0 → + 52.6 (H_2O; *c.*8). Anhydrous β-isomer; m p , 252°C.; $[\alpha]_n^{20}$ = + 34.9 → + 55.1 (H_2O; *c.*4) Not fermentable by ordinary yeasts; fermented by lactose yeasts.

Identification Phenylosazone; oxidation and hydrolysis with nitric acid to mucic acid; benzylphenylhydrazone

Occurrence. Found in the milk of all mammals to the extent of approximately 5 per cent. Authenticated examples of the existence of lactose in the plant kingdom are lacking.

[49] B. Helferich and J. F Leete, *Organic Syntheses*, *22*, 53 (1942).
[50] D. D. Reynolds and W. L. Evans, *J Am Chem. Soc* , *60*, 2559 (1938); B Helferich and W. Klein, *Ann* , *450*, 219 (1926).
[51] W Charlton, W N. Haworth and W J. Hickinbottom, *J Chem. Soc.*, 1527 (1927); W. N. Haworth and B Wylam, *ibid.*, *123*, 3120 (1923)
[52] E. O Whittier. *Chem Revs.*, *8*, 85 (1925-26).

Preparation.[53] Whey, obtained as a byproduct in the manufacture of cheese, upon evaporation deposits crystalline lactose, which is easily recrystallized from water.

The monohydrate of the alpha isomer crystallizes from solutions at temperatures below 93–95°C. and the more soluble beta isomer from aqueous solutions above this temperature.[54]

Structure. The disaccharide, after acid or enzymic hydrolysis (β-galactosidase), gives one molecule each of galactose and glucose. If the sugar is first oxidized with bromine to lactobionic acid and then hydrolyzed, gluconic acid and galactose are the products obtained.[55] This evidence establishes lactose as being a glucose galactoside.

After methylation and hydrolysis, the disaccharide yields a trimethylglucose and a tetramethylgalactose. As the tetramethylgalactose is the same as that obtained by a similar procedure from methyl galactopyranoside, it must be 2,3,4,6-tetramethylgalactose. The trimethylglucose is identical with that from maltose and is 2,3,6-trimethylglucose. Since the open-chain lactobionic acid after methylation and hydrolysis gives tetramethylgluconic "γ" lactone, the disaccharide linkage must be connected to carbon 4 of the glucose molecule[56] (see under Maltose). The principal evidence for the configuration of the glycosidic linkage rests on the known specificity of the galactosidases of almond emulsin. The sugar is hydrolyzed by both crude and purified almond emulsin and the relative rate of hydrolysis by the two emulsins is proportional to the β-galactosidase and not to the α-galactosidase content. As the enzyme studies indicate the existence of a β-galactosidic linkage, lactose may be described as 4-D-glucose β-D-galactopyranoside. Additional support is given by the synthesis of lactose by a method ordinarily giving β-galactosides.

Synthesis. The synthesis of lactose by the condensation of glucose and galactose at high temperatures in the presence of acetic anhydride or zinc chloride has been reported.[57] Fischer and Armstrong[58] obtained a glucose galactoside by the reaction of tetraacetylgalactosyl chloride, sodium ethylate and an aqueous solution of glucose. They obtained an osazone which they considered to be melibiose osazone. The properties of the osazone have since made it probable that the product is lactose.[59] A synthe-

[53] See. F. P. Nabenhauer, *Ind. Eng. Chem.*, *22*, 54 (1930).

[54] C. S. Hudson, *J. Am. Chem. Soc.*, *30*, 1767 (1908); J. Gillis, *Rec. trav. chim.*, *39*, 88, 677 (1920); R. W. Bell, *Ind. Eng. Chem.*, *22*, 51 (1930); W. E. Stringer, *Food Ind.*, *11*, 72, 262 (1939); P. F. Sharp and D. B. Hand, U. S. Patent, 2,182,618, Dec. 5, 1940.

[55] E. Fischer and J. Meyer, *Ber.*, *22*, 361 (1889).

[56] W. N. Haworth and C. W. Long, *J. Chem. Soc.*, 544 (1927)

[57] E. Demole, *Ber.*, *12*, 1035 (1879); Berthelot, *Bull. soc. chim.*, [2], *34*, 82 (1880); A. Pictet and H. Vogel, *Helv. Chim. Acta*, *11*, 209 (1928).

[58] E. Fischer and E. F. Armstrong, *Ber.*, *35*, 3144 (1902).

[59] H. H. Schlubach and W. Rauchenberger, *Ber.*, *59*, 2102 (1926).

sis of more importance in providing information on the structure of lactose involves as the first step the reaction of tetraacetylgalactosyl bromide and 2,3-isopropylidene-1,6-anhydromannopyranose in an organic solvent and in the presence of silver carbonate (Koenigs-Knorr reaction).[60] Since the only unsubstituted hydroxyl is at carbon 4 of the anhydromannose, the condensation must take place at this point. After removal of the isopropylidene (acetone) group, the product is acetylated, and the anhydro ring is opened by the action of sulfuric acid in glacial acetic acid and acetic anhydride. The resulting substance is a disaccharide octaacetate epimeric with lactose and, hence, called epilactose. This substance is then converted to lactose through the glycal synthesis by oxidation of lactal with perbenzoic acid. In all probability, the initial condensation produces a β-galactosidic linkage since tetraacetylgalactosyl bromide condenses with alcohols with the formation of β-galactosides.

2,3-Isopropylidene-1,6-anhydromannopyranose + tetraacetylgalactosyl bromide
$\xrightarrow{Ag_2CO_3}$ 4-(2,3 isopropylidene-1,6-anhydromannopyranose) tetraacetyl-β-
D-galactoside $\xrightarrow{HOAc}$ 4-(1,6-anhydromannopyranose) tetraacetyl-β D galactoside
$\xrightarrow{(Ac)_2O}$ 4 (diacetyl 1,6 anhydromannopyranose) tetraacetyl β D galactoside
$\xrightarrow[HOAc]{H_2SO_4 (Ac)_2O}$ 4-(tetraacetylmannose) tetraacetyl β-D-galactoside $\xrightarrow[HOAc]{HBr}$
4-(triacetylmannosyl bromide) tetraacetyl β-D galactoside $\xrightarrow[HOAc]{Zn}$ hexaacetyl
lactal $\xrightarrow{perbenzoic acid}$ hexaacetyllactose $\xrightarrow{OH}$ lactose

Enzymic syntheses of lactose from glucose have also been carried out.[61] Mammary tissue and other tissues not only catalyze the condensation of two molecules of hexose but also the transformation of glucose to galactose. It is possible that the biological formation of galactose from glucose may not be direct but may proceed from some such intermediate as lactic acid.[62]

The tolerance of normal humans for the oral administration of lactose is considerable. Urinary excretion takes place mainly after hydrolysis to glucose and galactose. When the sugar is injected intravenously into rabbits, it is excreted unchanged.[63]

It is reported that rats are unable to survive on a diet in which lactose is the sole source of carbohydrate. On such a diet, the rats develop diarrhea and alopecia and finally die.[64]

[60] W. T. Haskins, R. M. Hann and C. S. Hudson, J. Am. Chem. Soc., 64, 1852 (1942).

[61] G. A. Grant, Biochem. J., 30, 2027 (1936); W. E. Petersen and J. C. Shaw, Science, 86, 398 (1937); D. Michlin and M. Lewitow, Biochem. Z., 271, 418 (1934).

[62] J C. Shaw, W. L. Boyd and W. E. Petersen, Proc. Soc. Exptl. Biol. Med., 38, 579 (1938).

[63] L. B. Winter, J Physiol., 71, 341 (1931)

[64] B. H. Ershoff and H J Deuel, Jr., J Nutrition, 28, 225 (1944).

The conditions for the maximum conversion of lactose to hexose sugars have been studied by Ramsdell and Webb.[64b] Using 0.007 M HCl as the hydrolyzing agent and a temperature of 147°, 30 per cent solutions of lactose are converted to hexose sugars to the extent of 93 per cent of the theory in less than 65 minutes. A mixture of equal parts of glucose and galactose is soluble in water to the extent of 42 per cent at 25°C.

Maltose
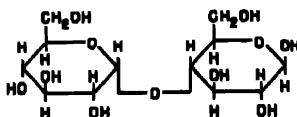
Synonyms. 4-D-Glucose α-D-glucopyranoside, 4-(α-D-glucopyranosyl)-D-glucose, malt sugar.

Properties. Obtained as a monohydrate of the beta isomer: m.p. 102 - 103°C.; $[\alpha]_D^{20} = + 111.7 \rightarrow + 130.4$ (H_2O; $c.4$). Fermentable by yeasts in the presence of dextrose.

Identification. Phenylosazone, β-naphthylhydrazone.

Occurrence. Maltose occasionally has been recorded as present in intact plants. However, since it is a product of the enzymic hydrolysis of starch and since both amylases and starch are found in the same plants, it may be a secondary product formed during the extraction process.

Preparation[12 65] Soluble starch, made from commercial starch by a mild treatment with acid, is hydrolyzed by the enzymes of barley flour to a mixture of maltose and dextrins. These are separated by fractional precipitation with alcohol and the crude maltose is recrystallized from aqueous alcohol. Commercial maltose contains considerable quantities of dextrins which are removed by fractional precipitation of an aqueous solution with alcohol.

Structure. The methylation of maltose leads to a methyl heptamethylmaltoside which by acid hydrolysis is converted to a crystalline tetramethylglucose and a trimethylglucose.[66] The crystalline tetramethylglucose is identical with that obtained by the hydrolysis of methyl tetramethylglucopyranoside and has the methyl groups at positions 2,3,4, and 6. The trimethylglucose does not form an osazone (methoxyl on carbon 2) and on methylation gives the well-known methyl 2,3,4,6-tetramethylglucopyranoside. Of the many possible trimethylglucoses, only three conform to

[64b] G. A. Ramsdell and B H Webb *J Dairy Sci*, **28**, 677 (1945)

[65] T. S. Harding, *Sugar*, **25**, 350 (1923); H C. Gore, U S. Patent, 1,557,079, Jan 21, 1928

[66] W N. Haworth, J V Loach and C W. Long, *J. Chem Soc.*, 3146 (1927)

these specifications. These are the 2,3,4-, the 2,3,6- and the 2,4,6-trimethylglucoses. The synthetic 2,3,4-trimethylglucose differs from the product of the hydrolysis of the methylated maltose; its structure is determined through its synthesis by the methylation of 6-tritylglucose and by its oxidation to *xylo*-trimethoxyglutaric acid. Since the trimethylglucose from the methylated maltose is oxidized by nitric acid to (dextro)-dimethyl-L-tartaric acid, it must be the 2,3,6-trimethylglucose. In agreement with this conclusion, the third possible isomer 2,4,6-trimethylglucose, which has been synthesized, gives neither of these dibasic acids when oxidized.

The identification of the trimethylglucose from maltose as the 2,3,6-trimethylglucose still leaves two possibilities for the structure of maltose since the disaccharide bridge may be connected to carbon 4 or carbon 5. The position of the linkage is shown by the bromine oxidation of maltose to maltobionic acid which on methylation and hydrolysis yields, in addition to tetramethylglucose, a tetramethylgluconic acid. Inasmuch as this acid forms a lactone identical with methylated gluconic "γ"-lactone, it must be 2,3,5,6-tetramethylgluconic acid with the unsubstituted hydroxyl on carbon 4 representing the position of the disaccharide linkage.[67]

The above evidence proves[68] that maltose consists of two glucose residues connected between carbons 1 and 4' by an oxygen bridge, but the configuration of the glucosidic linkage remains to be determined. This determination is particularly necessary, for another important disaccharide, cellobiose, gives exactly the same final products as outlined above for the maltose. The best proof of the configurations of the glucosidic carbon of these two disaccharides is obtained from studies of the enzymic hydrolysis. Maltose is hydrolyzed by the same yeast enzyme (α-glucosidase) as that which hydrolyzes methyl α-glucoside. The β-glucosidase of almond emulsin produces no significant cleavage. Cellobiose, however, is hydrolyzed by the same enzyme (β-glucosidase) as that acting on β-glucosides. From this evidence, maltose is given the formula of 4-glucose α-D-glucopyranoside and cellobiose the formula 4-glucose β-D-glucopyranoside. These formulas are given confirmation by the high *dextro* optical rotation of maltose and the small rotation of cellobiose. As a rule, the α-glucosides are strongly dextrorotatory and the β-glucosides levorotatory.

Isomaltose

The unfermentable fraction of the products formed by the action of acids on glucose or on starch has been given the name of isomaltose.[69] The

[67] W. N. Haworth and S. Peat, *J. Chem. Soc.*, 3094 (1926).
[68] W. N. Haworth, C. W. Long and J. H. G. Plant, *J. Chem. Soc.*, 2809 (1927).
[69] E. Fischer, *Ber.*, 28, 3024 (1895).

nature of the isomaltose is of considerable interest because the mother liquors ("hydrol") from the preparation of D-glucose from starch contain considerable quantities of nonfermentable material. The product obtained is very difficult to purify. Although a crystalline osazone is reported by Fischer, the osazones obtained by other workers have appreciably different properties. Methylation studies of the isomaltose show that the bulk of the material is one or both of the 6-glucose glucosides, for hydrolysis of the methylated derivatives gives 2,3,4-trimethylglucose and 2,3,4,6-tetramethylglucose. Although it has been suggested[70] that isomaltose is 6-glucose α-glucoside, the lack[71] of action by yeasts and yeast enzymes (α-glucosidase and invertase) would be better explained by the gentiobiose (6-glucose β-glucoside) structure. This explanation would also agree with the isolation[72] of gentiobiose (as the octaacetate) from the "hydrol," acetylated after removal of fermentable material. However, until additional evidence has been obtained, the homogeneity of the product remains very questionable.

Melibiose
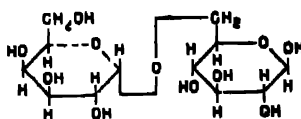
Synonyms 6-(Glucose α-D-galactopyranoside, 6-(α-D-galactopyranosyl)-D-glucose

Properties. Crystallizes as the dihydrate of the beta isomer, m.p., 82-85°C ; $[\alpha]_D^{20} = +111.7 \rightarrow +129.5$ (H₂O; c,4). Fermentable by bottom yeasts but not by most top yeasts.

Identification. Phenylhydrazone and osazone. hydrolysis of the sugar or osazone and oxidation of the galactose to mucic acid (lactose gives the same reaction).

Occurrence. The sugar is found as a component of the trisaccharide raffinose and has been found free in plant exudations.

Preparation.[43,7] Raffinose, a trisaccharide found in beet molasses, is hydrolyzed by invertase since it contains a sucrose linkage; melibiose and fructose are produced. By using baker's yeast (top yeast), hydrolysis and simultaneous removal of fructose by fermentation takes place. The sirup crystallizes directly or after purification through the octaacetate.

Structure and Synthesis. Hydrolysis of methylated melibionic acid gives

[70] K. Myrbäck, *Svensk Kem. Tid.*, **53**, 264 (1941)

[71] A. Georg, *Compt. rend. soc. phys. hist. nat. (Genève)*, **47**, 94 (1930).

[72] H. Berlin, *J. Am. Chem. Soc.*, **48**, 1107, 2627 (1926)

[73] C. S. Hudson and T. S. Harding, *J. Am. Chem. Soc.*, **47**, 2731 (1915), T. S. Harding, *Sugar*, **25**, 514 (1923).

tetramethylgluconic acid and a tetramethylgalactose. The former is the 2,3,4,5-isomer since it does not form lactones and since it is oxidized by nitric acid to tetramethylglucaric acid. The tetramethylgalactose is identical with that obtained by the hydrolysis of methyl tetramethylgalacto-pyranoside and must be 2,3,4,6-tetramethylgalactose. The disaccharide linkage must connect carbon 1 of the galactose and carbon 6 of the glucose residue.[86]

When tetraacetylgalactosyl bromide condenses with 1,2,3,4-tetra-acetylglucose in the presence of silver carbonate, a disaccharide octaacetate is formed[74] which is different from the melibiose octaacetates and which may be identical with the so-called allolactose isolated from milk. This compound probably has a β-galactosidic linkage, inasmuch as β-galactosides are the customary products of the Koenigs-Knorr reaction as applied here, and is 6-glucose β-D-galactoside. When quinoline is employed to aid in the condensation of tetraacetylgalactosyl bromide and phenol, some phenyl α-galactoside is formed. The replacement of silver carbonate by quinoline in the disaccharide synthesis leads to the preparation[75] of melibiose octa-acetate, which probably has an α-galactosidic linkage because it differs from the product obtained from the Koenigs-Knorr synthesis

Sucrose
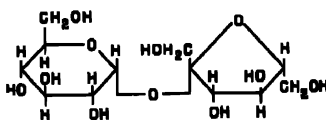
Synonyms. Saccharose, cane sugar, beet sugar, 1 α-D-glucopyranose β-D-fructofuranoside, 2-β-D-fructofuranose α-D-glucopyranoside.

Properties. $[\alpha]_D^{20} = + 66.53$ (H$_2$O; c,26); m.p., 160° to 186°C., depending on the medium used for purification.[76] Nonreducing. Fermentable by yeasts.

Identification. An alkaline solution of diazouracil turns green only in the presence of sucrose or of oligosaccharides containing sucrose as a component, i.e., raffinose, gentianose and stachyose.[77]

Occurrence. The sugar occurs almost universally throughout the plant kingdom in the juices, seeds, leaves, fruits, flowers and roots of plants. Sucrose was reported in all of the 281 species of phanerogams studied by Bourquelot and his associates.[78] Honey consists principally of sucrose and

[74] B. Helferich and H. Rauch, *Ber* , *59*, 2655 (1926)

[75] B. Helferich and H Bredereck, *Ann* , *465*, 166 (1928)

[76] See: A. Pictet and H. Vogel, *Helv. Chim. Acta*, *11*, 901 (1928)

[77] H. Raybin, *J. Am. Chem. Soc.*, *59*, 1402 (1937).

[78] Quoted by C. Béguin, *Pharm. Acta Helv.*, *1*, 90 (1926).

its hydrolysis products glucose and fructose (invert sugar). The principal sources of commercial interest are sugar cane, sugar beets and the sap of maple trees.

Manufacture. Cane Sugar. Sugar cane (*Saccharum officinarum* L.) is a species of the family of grasses having a single stalk and often reaching a height of 18 feet. During harvesting, the cane is cut close to the ground and topped. In order to prevent losses due to the hydrolysis (inversion) of the sucrose, the stalks are processed as rapidly as possible. This is accomplished by first passing them through cutting machines and then through roll crushers which force out the juice. The pressed cane fibre (called bagasse), may be extracted with water and passed a second time through the rollers. The bagasse may be used as a fuel or for the preparation of products such as certain types of wall board. Although the juice varies considerably in composition, the following analysis may be taken as being representative.

Water	83.0%
Sucrose	15.0%
Reducing sugars	1.0%
Other organic material	0.5%
Ash	0.5%

The juice, originally acidic, is made slightly alkaline by the addition of lime which acts to prevent hydrolysis of the very acid-sensitive sucrose and which also removes many impurities. This purification by the use of lime, called defecation, is the principal purification process in the preparation of the raw sugar. When the alkaline juice is heated, a heavy scum or cake which forms on the surface contains many of the impurities while still others settle out on the bottom. After separation of the impurities, clear juice is drawn into evaporating pans.

Subsequent to a preliminary evaporation of the purified juice in vacuum pans to a solution of about 50 per cent solids, the sirup is transferred to vacuum pans in which the rate of evaporation and the temperature may be accurately controlled. The evaporation is continued until crystals appear, and then fresh sirup is added at a rate such that the original crystals grow without the formation of new crystal nuclei (false grain). When the desired growth has been obtained, the mass of crystals and sirup, called the massecuite, is dropped into centrifuges; the mother liquor is separated; and the crystals are washed with clear juice and finally removed. These crystals constitute the "raw sugar" of commerce and are the raw material for the refinery. Successive crops of crystals of decreasing purity are taken from the mother liquors until no more may be economically obtained. The final mother liquor (named "blackstrap") is a dark-colored viscous liquid which

is sold as cattle food and which is also extensively employed as the source of carbohydrates in the preparation of industrial alcohol and rum since it still contains 50 per cent of fermentable sugar.

The "raw sugar," as shipped to the refineries, is usually brown in appearance, and the principal task of the refinery is to remove this color and to make a nonhygroscopic, smooth-pouring product. As a first step the crystals are washed in centrifugals with the mother liquors obtained in previous refinery operations. Considerable color is removed without dissolving the crystals. The washed raw sugar is dissolved in a small amount of hot water; a little lime is added and the solution is purified by passage through towers of activated animal charcoal (bone black) or vegetable charcoal. The filtered solution is concentrated in vacuum pans as described for the raw sugar, and the crystalline refined sugar is obtained. This last stage of the process is difficult, for all the various factors involved in obtaining a product with a predetermined, uniform particle-size have not been discovered. Several more crops of white crystals may be obtained from the mother liquors before "brown sugars" are obtained. The brown crystals from the mother liquors of the refinery operations, and not the brown "raw sugar," are the "brown sugars" of commerce

Beet Sugar. In other than tropical and sub-tropical countries, the sugar beet (*Beta vulgaris*) is the principal source of sucrose. After harvesting, the beets are taken to the mill, washed and cut into slices called "cosettes." The cosettes are delivered from a central spout into a series of diffusion vessels. Here they are extracted with hot water utilizing the counter-current principle. Fresh water passes first into the diffuser having the most exhausted charge; the solution then goes through the diffusion vessels in order of increasing sugar content and finally passes through the fresh charge of cosettes. The dark diffusion juice containing about 12 per cent of sucrose is agitated with lime for several hours. Carbon dioxide is passed into the solution, and the precipitate, which contains most of the impurities, is separated by filtration. The light-yellow filtrate is decolorized by a treatment with sulfur dioxide and after a final filtration is concentrated in multiple-effect vacuum pans. The crystals are developed during the evaporation in the same manner as for the cane sugar and are then separated from the mother liquors by centrifugation and finally dried. The evaporation and crystallization are carried out repeatedly with the mother liquors as long as enough sugar is obtained to make the process economical.

Additional quantities of sucrose may be obtained from the molasses by diluting it to a concentration of about 7 per cent sugar, cooling to 12°C. and adding lime (Steffen process). A difficultly soluble compound of sucrose with three moles of lime, tricalcium saccharate, crystallizes. The tricalcium saccharate, after separation from the final molasses, serves in the

place of lime for the purification of the warm diffusion juice. Some beet sugar factories recover additional sugar from the molasses by a treatment with barium hydroxide, which forms the difficultly soluble barium saccharate. This saccharate is decomposed with carbon dioxide, and the insoluble barium carbonate is separated from the sucrose. The barium carbonate is reclaimed and reconverted to barium hydroxide. The final molasses is usually sold for cattle food or for industrial fermentations particularly when mixed with "blackstrap" molasses.

The sugar may be purified by recrystallization from aqueous or aqueous alcohol solutions.[79]

Structure. The sugar is hydrolyzed by acids and by enzymes to a mixture of equal amounts of fructose and glucose. The process is called *inversion* since the optical rotation changes from dextro to levo because of the high levorotation of the fructose. The mixture formed is called *invert sugar.* Octamethylsucrose, obtained by the methylation of sucrose, does not undergo inversion of rotation on hydrolysis, and two dextrorotatory tetramethylhexoses are obtained. The tetramethylglucose is the well known 2,3,4,6-tetramethylglucose. The structure of the fructose derivative is shown by the following evidence. (For the structure of tetramethylfructo-pyranose, see p. 209.) Oxidation with nitric acid gives a liquid trimethyl-2-ketogluconic acid, which in turn is oxidized by acid permanganate to a crystalline trimethyl-D-arabonic γ-lactone. This lactone is identical, except for the sign of the rotation, with the product obtained by the oxidation of trimethyl-L-arabinose. Inasmuch as the trimethyl-D-arabonic lactone from sucrose yields (levo)dimethyl-D-tartaric acid (I) on further treatment with

$$\begin{array}{ccc} & \text{H} & \text{OCH}_3 \\ \text{HOOC} & \text{C} \text{---} \text{C} \text{---} \text{COOH} & \text{(I)} \\ & \text{OCH}_3 \ \text{H} \end{array}$$
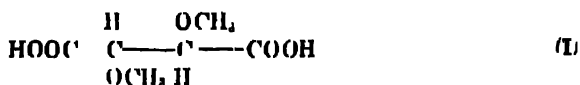
nitric acid, it must have the methyl groups at positions 2,3 and 5; the original methylated fructose from sucrose is the 1,3,4,6-tetramethyl-fructofuranose. If the probable assumption is made that the sucrose has ring structures for the component sugars, then the connection between the hexose units must be between the anomeric carbons and sucrose has the formula given above.[80]

The above structure is confirmed by the results obtained by periodic acid oxidation.[81] Sucrose consumes three moles of periodic acid, and one

[79] F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars", Circular C440 of the National Bureau of Standards; Washington, D. C. (1942).

[80] J. Avery, W. N. Haworth and E. L. Hirst. *J. Chem. Soc.,* 2308 (1927); W. N. Haworth, E. L. Hirst and A Learner, *ibid.,* 2432 (1927)

[81] P. Fleury and J Courtois, *Bull. soc. chim ,* [5]. *10,* 245 (1943); *Compt. rend.,* *216,* 65 (1943).

mole of formic acid is formed. After bromine oxidation of the tetraaldehyde and subsequent hydrolysis, hydroxypyruvic, D-glyceric and glyoxylic acids are obtained. (For a discussion of this method see p. 209.)

The configuration of the glycosidic linkages of sucrose is probably alpha for the glucose component and beta for the fructose component. The hydrolysis of sucrose by yeast α-glucosidase and not by the β-glucosidase of almond emulsin supports the α-glucoside structure. Similarly, the hydrolysis of the sugar by yeast invertase, an enzyme which hydrolyzes beta but not α-fructofuranosides, supplies evidence[52] for the β-fructofuranoside structure. Comparisons made by use of the Isorotation rules agree with the above evidence because only the α,β-configuration gives agreement between the calculated and observed rotations for sucrose and sucrose octaacetate.[51]

Synthesis. The chemical synthesis of sucrose remains one of the outstanding unsolved problems of sugar chemistry. Many attempts have been made, but it is generally agreed that the aim has never been achieved since it has not been possible[54] to reproduce the single reported chemical synthesis. The problem is complicated because of the theoretical existence of four isomers having sucrose structures but having different configurations for the anomeric carbons. These may be designated as the α-α, β-β, α-β and β-α isomers. Sucrose is probably the α-β isomer (α-glucosidic, β-fructosidic linkage), and the methods of synthesis employed usually result in the synthesis of the β-α isomer (isosucrose). In some methods the number of possible isomers is further increased since either of the reacting molecules may condense with others of the same kind to form difructosides and diglucosides each of which should exist in four α,β-isomers. Still another hindrance arises from the difficulty of obtaining well-defined suitable derivatives of fructofuranose.

Attempts at the synthesis of sucrose have been made by Pictet and Vogel,[55] Irvine and associates[56] and Klages and Niemann.[53] The 1,3,4,6-tetraacetylfructosyl chloride and 2,3,4,6-tetraacetylglucose react in the presence of silver carbonate giving a disaccharide octaacetate isomeric with sucrose and called isosucrose. The isosucrose probably has a β-glucosidic, α-fructosidic structure: 2-β-glucopyranosyl-α-fructofuranoside. However, Schlubach and Middelhoff[57] report that isosucrose exhibits reducing prop-

[52] H. H. Schlubach and G. Rauchalles, *Ber.*, **58**, 1842 (1925); C. B. Purves and C. S. Hudson, *ibid.*, **59**, 49 (1937)

[53] F. Klages and R. Niemann, *Ann.*, **529**, 185 (1937).

[54] G. Zemplén and A. Gerecs, *Ber.*, **62**, 981 (1929); A. Georg, *Helv. Chim. Acta, 16*, 130 (1933).

[55] A. Pictet and H. Vogel, *Helv. Chim. Acta, 11*, 436 (1928); *Ber.*, **62**, 1418 (1929)

[56] J. C. Irvine, *et al*, *J. Am. Chem. Soc.*, **51**, 1279, 3609 (1929); **54**, 1079 (1932); **57**, 1411 (1935).

[57] H. Schlubach and B. Middelhoff, *Ann*, **550**, 134 (1942)

erties which would be best explained by the presence of a free reducing group. But inasmuch as the reduction of Fehling solution by isosucrose is much slower[88] than that by maltose, it seems probable that reduction may be a consequence of a lability of the sugar to alkaline hydrolysis such as has been observed for turanose and some glycosides (see p. 199). In attempts to condense tetraacetylglucosyl halides with fructose derivatives having an unsubstituted hydroxyl group at carbon 2, no disaccharide formation could be detected. The action of phosphorus pentoxide on an anhydrous solution of 2,3,4,6-tetraacetylglucose and tetrabenzoyl or tetraacetyl fructofuranose results in the combination of two molecules of the glucose derivative with the formation of 1-(β-D-glucose) β-D-glucoside (β,β-trehalose) derivatives.

Numerous reports of the biochemical synthesis of sucrose have been made. Invert sugar solutions (equal amounts of fructose and glucose) are partially transformed to sucrose by the simultaneous action of invertase and phosphorylase in the presence of inorganic phosphate.[89] The *in vivo* synthesis probably takes place from glucose by a phosphorylation process since fructose and glucose phosphates are in equilibrium in living organisms.[90] Both glucose and fructose phosphates are found in the leaves of sugar beets, and potato slices synthesize sucrose when placed in glucose or fructose solutions.[91] Many cut plants, immersed in sugar solutions in the dark, produce sucrose from dissolved glucose, fructose, galactose and maltose, although dihydroxyacetone, xylose and glycerol are not utilizable.[92] Experiments with detached blades, sheaths, stems and roots of sugar cane indicate that the formation of glucose and fructose is preliminary to the synthesis of sucrose since glucose and fructose are converted to sucrose by the leaves in the dark by a process involving phosphorylation.[93]

Hassid, Doudoroff and Barker made a notable contribution when they found that a bacterial emulsin (*Pseudomonas saccharophila*) contains a phosphorylase which hydrolyzes sucrose to fructose and glucose 1-phosphate in the presence of inorganic phosphates and which synthesizes sucrose from the latter products. From the e reaction products, *crystalline* synthetic sucrose has been obtained for the first time. Analogous disaccharides are formed from glucose 1-phosphate and sorbose or D-xylulose.[94]

[88] A. Georg, *Ann.*, *561*, 272 (1942).

[89] A. Oparin and A. Kurssanow, *Biochem. Z.*, *239*, 1 (1931).

[90] J. Burkard and C. Neuberg, *Biochem. Z.*, *270*, 229 (1934).

[91] J. M. Nelson and R. Auchincloss, *J. Am. Chem. Soc.*, *55*, 3709 (1933).

[92] M. Nurmia, *Ann. Acad. Sci. Fennicae*, *44 A*, No. 8 (1935).

[93] C. E. Hartt, *Hawaiian Planters' Record*, *41*, 33 (1937); *47*, 113, 155 (1943); *48*, 31 (1944).

[94] W. Z. Hassid, M. Doudoroff and H. A. Barker, *J. Am. Chem. Soc.*, *64*, 1416 (1944); M. Doudoroff, *Federation Proc.*, *4*, 241 (1945); W. Z. Hassid, M. Doudoroff H. A. Barker and W. H Dore, *J. Am Chem Soc*, *68*, 1465 (1946)

α,α-Trehalose
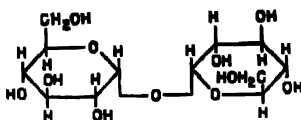
Synonyms 1-(α-D-Glucopyranose) α D glucopyranoside, mycose, mushroom sugar.

Properties of Dihydrate. Nonreducing; m.p., 97°C., $[\alpha]_D^{20} = + 178.3$ (H$_2$O; *c*,7). Fermentable by most yeasts.

Identification. Hexaacetate

Occurrence.[95] The sugar was originally separated from rye ergot and is a common constituent of fungi. It is found in young mushrooms, but as the plants develop the trehalose content is replaced by mannitol, and in aged or dried mushrooms the sugar is completely replaced by mannitol.[96] Trehala manna, a source of trehalose, is not a true manna (plant secretion) but consists of an oval shell about the size of an olive formed by certain insects found in Syria. Probably the best source of the sugar is the "resurrection plant," *Selaginella lepidophylla*, a common plant of the southwestern United States, which contains free trehalose. As some workers have not been able to obtain the sugar from this source, it is probable that the harvesting time must be carefully controlled. Sea weeds are also reported to contain considerable quantities of the disaccharide.

Preparation.[96] The sugar is extracted from trehala manna by the action of boiling 75 per cent alcohol. After concentration, the extracts are purified by treatment with basic lead acetate and the excess lead removed with hydrogen sulfide. The filtered solution, after concentration, deposits crystals of trehalose. Essentially the same process is employed in obtaining the sugar from coarsely ground *Selaginella*, but the extraction may be carried out with water rather than alcohol.

Hungarian ergot may be used as a source.[97] The benzene-extracted ergot is treated with alcohol. The extracts are purified with activated carbon and evaporated; crystalline material is obtained by diluting the residue with aqueous alcohol and allowing the solution to crystallize.

Structure. Methylation and hydrolysis of trehalose produce two moles of tetramethylglucopyranose.[98] The sugar consumes four moles of periodic acid and two moles of formic acid are formed.[99] Hence, the disaccharide

[95] T. S. Harding, *Sugar*, **25**, 476 (1923).

[96] E. Bourquelot, *Compt rend.*, *111*, 578 (1890).

[97] G. Zemplén, *Chem. Abst.*, *31*, 6204 (1937).

[98] H. Schlubach and K Maurer, *Ber.*, *58*, 1179 (1925); (the 2,3,5,6-tetramethylglucose of S. and M. is the compound now known as 2,3,4,6-tetramethylglucose)

[99] E. L. Jackson and C. S. Hudson, *J Am. Chem. Soc.*, *61*, 1530 (1939).

must have a pyranose structure for each glucose component and have a glycosidic linkage connecting the two anomeric carbons. Consideration of the optical rotatory relationships indicates that both anomeric carbons have the alpha configuration.

Attempts at the synthesis of the compound have been unsuccessful but β,β and α,β-isomers have been obtained[98] (see under Sucrose).

Turanose[100a]
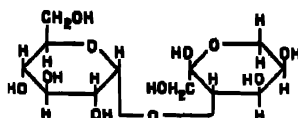
Synonyms. 3-Fructose α-D-glucopyranoside, 3-(α-D-glucopyranosyl)-D-fructose.

Properties. m.p., $157°C.$; $[\alpha]_D^{20} = +27.3 \rightarrow +75.8$ (H_2O; $c,4$). Not fermented by yeasts.

Identification. Phenylosazone.

Occurrence. The trisaccharide melezitose yields on partial hydrolysis turanose and D-glucose.

Preparation.[100b] Melezitose is partially hydrolyzed by dilute sulfuric acid with the liberation of glucose and turanose. The glucose is removed by fermentation and the turanose directly crystallized.

Structure. As shown by G. Tanret, the sugar is hydrolyzed to D-fructose and D-glucose by yeast α-glucosidase and hence must be an α-glucoside. This conclusion receives confirmation from the lack of hydrolysis of the disaccharide by almond emulsin[101] The above evidence and the resistance of the sugar to oxidation by alkaline hypoiodite solutions, coupled with its reduction of alkaline copper-salt solutions, show that the unsubstituted hemiacetal group belongs to the fructose component. The mutarotation of turanose resembles that of fructose and probably is caused by an interconversion between pyranose and furanose isomers rather than between alpha-beta isomers.[102] Hence, the hydroxyls of carbons 5 and 6 of the fructose component must be unsubstituted. The formation of an osazone proves that free hydroxyls are present on both carbons 1 and 2.[101 103] This evidence eliminates all positions except carbons 3 and 4 for the disaccharide linkage. If the linkage involved carbon four of the fructose residue,

[100a] C. S. Hudson, *Advances in Carbohydrate Chem*, *2*, 1 (1946)

[100b] C. S. Hudson and E. Pacsu, *J. Am. Chem Soc*, *52*, 2522 (1930).

[101] M. Bridel and T Aagaard, *Compt. rend*, *184*, 1067 (1927); T. Aagaard, *Chem Abst.*, *24*, 1089 (1930).

[102] H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, *20*, 773 (1938).

[103] E. Pacsu, E J Wilson, Jr , and L. Graf, *J. Am. Chem. Soc.*, *61*, 2675 (1939)

the turanose would yield the same osazone as maltose, but actually the two osazones are quite different.[102] Hence, the two monosaccharides must be connected by an oxygen bridge between carbon 3 of the fructose and carbon 1 of the glucose component. It is, then, 3-fructose α-D-glucopyranoside. This structure is confirmed by the ease of hydrolysis of the sugar by alkalies[104] and by the formation of tritritylturanose.[105]

By an ingenious application of stereochemical principles, the structure of turanose has been related to that of maltose, and a direct proof of its structure has been obtained.[106] The proof involves the conversion of maltose to epimaltose by means of the glycal synthesis (see p. 128), and the reduction of the epimaltose to the same product (3 or 4-α-glucosylmannitol) as that obtained by the reduction of turanose. The symmetry of mannitol is such that substitutions at the 3 and 4 positions of mannitol are equivalent substitutions. An outline of the important steps in the synthesis are given in the accompanying formulas.

Maltose

Epimaltose

4 α Glucosyl-D mannitol

(These two products are identical)

(Gl = α-glucosyl group)

Turanose

3-α-Glucosyl-D mannitol
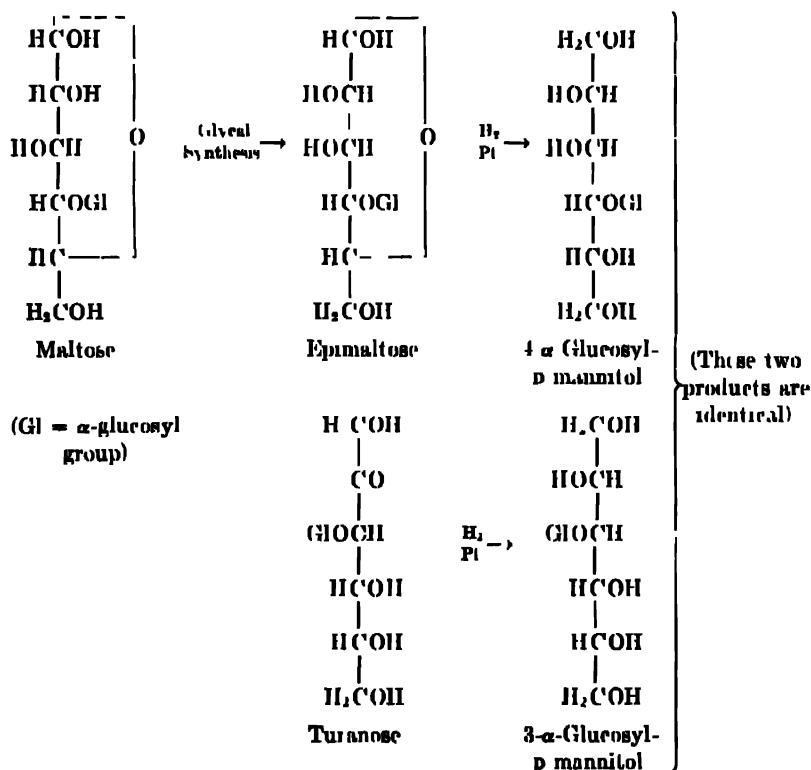
[104] H S Isbell, J Research Natl Bur Standards, 26, 35 (1941)
[105] E Pacsu, J Am Chem Soc , 53, 3099 (1931)
[106] C S Hudson, J Org Chem , 9, 117, 470 (1944)

B. Tri- and Tetrasaccharides

Gentianose

Gentiobiose   Sucrose
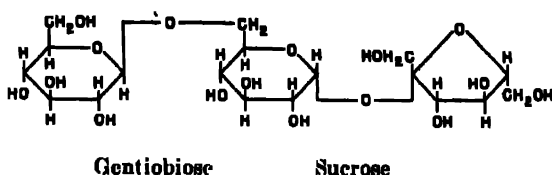
Synonyms 1-(6-D-Glucopyranosyl-α-D-glucopyranose) β-D-fructofuranoside.
Properties. m.p., 209–211°C.; $[\alpha]_D^{20} = +31.5$ (H_2O). Nonreducing.
Occurrence. The sugar is found in the rhizomes of many species of *Gentian*.
Preparation.[107] Powdered gentian root is extracted with 90 per cent alcohol and the sugar isolated from the extracts.
Structure. Gentianose yields two moles of D-glucose and one of D-fructose when completely hydrolyzed by acids. Partial acid hydrolysis or the action of invertase produces fructose and gentiobiose.[108] The enzymes of almond emulsin cleave the disaccharide into glucose and sucrose. This evidence suffices to fix the structure of the trisaccharide. The two glucose units must be connected by a 1,6′ β-glucosidic linkage. The fructose unit must form one end of the molecule, be connected to the reducing carbon of the gentiobiose component through a sucrose linkage and have a furanose structure.

Melezitose[100a]
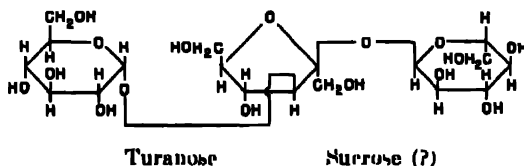
Turanose   Sucrose (?)

Synonyms. (α-D-Glucosyl)-sucrose; 2-[3-(α-glucopyranosyl)-β(?)-D-fructofuranose] α-D-glucopyranoside.
Properties of Dihydrate. m.p., 153–154°C ; $[\alpha]_D^{20} = +88.2$ (H_2O; c, 4). Not fermented by top (baker's) yeasts.
Identification. Hendecaacetate.
Occurrence. The sugar, discovered by Berthelot in 1859, is a constituent of the sweet exudations of many plants such as the "honey dew" of limes and poplars and the manna exuded from insect-produced wounds of the Douglas fir, Virginia pine, larch, etc. In dry seasons when the supply of flower nectar is insufficient, bees may collect these mannas or honey dews, and the honeys may contain considerable quantities of melezitose.[109] When

[107] M Bridel and M. Desmarest, *J. pharm. chim*, **9**, 485 (1929)
[108] E. Bourquelot and H Hérissey, *Compt rend*, **135**, 399 (1902)
[109] C. S. Hudson and S. F. Sherwood, *J. Am. Chem. Soc*, **42**, 116 (1920)

the quantity of the trisaccharide is great, crystallization of the honey may take place in the comb. Probably because of the resistance of the melezitose to hydrolysis by invertase, honeys which contain this sugar will not serve as foods for bees.

Preparation. If available, the melezitose-rich honey provides the best source since the crystallized sugar is easily separated by dilution of the honey with alcohol and centrifugation.[79,109]

Mannas from various sources may be utilized by extracting the impurities with aqueous alcohol and then extracting the trisaccharide with water.[110] The sugar crystallizes from the aqueous extracts after the addition of alcohol.

Structure. Complete acid hydrolysis leads to the formation of one mole of D-fructose and two moles of D-glucose.[110] Dilute acids hydrolyze the sugar to glucose and a disaccharide, turanose, and the ease of hydrolysis is about the same as that of sucrose.[100b] Since turanose is 3-fructose α-D-glucopyranoside (see under Turanose), the nature of one of the disaccharide linkages is established. The lack of reducing power of melezitose proves that the second linkage is between the reducing carbons of the second glucose unit and the fructose portion of the turanose unit. Both disaccharide linkages are hydrolyzed by α-glucosidase[111] and must have the alpha configuration in relation to the glucose units. The configuration of the fructosidic linkage is still undetermined but in all probability is beta, if the configuration is beta, the second disaccharide component may be sucrose. The lack of action by invertase is to be ascribed to the substitution (at carbon 3) of the fructose unit of sucrose by a second glucose unit.

Raffinose

Melibiose   Sucrose
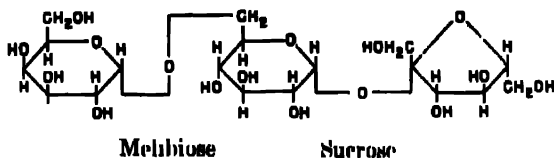
Synonyms. Gossypose, melitose, melitriose, (α-galactosyl)-sucrose, 1-[6-(α-galactopyranosyl)-α-D-glucopyranose] β-D-fructofuranoside.
Properties of Pentahydrate. m.p., 80°C.: $[\alpha]_D^{20} = + 105.2$ (H₂O; c, 4).
Partially fermented by top yeast (baker's yeast) with the formation of melibiose; completely fermented by bottom yeast. Nonreducing.
Identification. Hendecaacetate.

[110] G. Tanret, *Bull. soc. chim.*, [3], **35**, 817 (1906).
[111] R. Weidenhagen, *Z. Ver. deut. Zucker-Ind.*, **78**, 751 (1928)

Occurrence. The sugar exists in a free condition but in very small amounts (0.01 to 0.02 per cent) in sugar beets and accumulates in the mother liquors during the preparation of beet sugar (sucrose). The beet molasses provides an excellent source for the sugar. It has been found in many plants of which cotton (cotton seeds) and Australian eucalyptus (manna) may be particularly mentioned.

Preparation.[112] The sugar is available as a by-product of the barium process for the recovery of sucrose from beet molasses (see under Sucrose) and crystallizes directly from the final molasses.[113] Cotton-seed meal may also be utilized by extracting the sugar with water, precipitating it as a difficultly soluble compound with calcium (or barium) hydroxide and removing the calcium by carbonation.[114]

Structure. Complete acid hydrolysis leads to the formation of one mole each of D-glucose, D-fructose and D-galactose. Mild acidic hydrolysis affects only one linkage, and melibiose and fructose result.[115] Inasmuch as invertase produces the same end products, a sucrose type of linkage must be present. The presence of a sucrose linkage is proved by the action of almond emulsin (α-galactosidase constituent) which produces sucrose and D-galactose.[116] This evidence fixes the order of the monosaccharides and of their method of combination since the disaccharide connections of sucrose and melibiose

(Melibiose portion)   (Sucrose portion)

α-galactosyl $\xrightarrow{1-6}$ α-glucosyl $-\xrightarrow{1'-2''}$ β-fructofuranoside

hydrolyzed by   hydrolyzed by
α-galactosidase   invertase
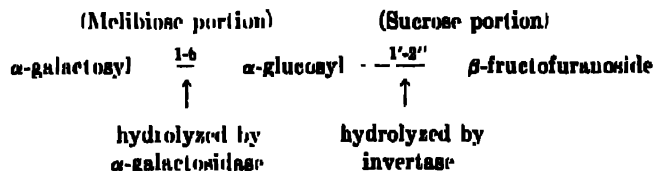
have been established (see discussion under these disaccharides). Hydrolysis of the methylated sugar gives the expected methylated monosaccharides, identical with those obtained from melibiose and sucrose.[117]

An isomer of raffinose, planteose, occurs in plantains (a common weed, *Plantago major*) and other *Plantago* species.[118] Acids hydrolyze it to galactose, glucose and fructose.

[112] T. S. Harding, *Sugar*, **25**, 308 (1923).

[113] E. H. Hungerford and A R. Nees, *Ind. Eng. Chem.*, **26**, 462 (1934).

[114] E. P. Clark, *J. Am. Chem. Soc.*, **44**, 210 (1922); D. T. Englis, R. T Decker and A. B. Adams, *ibid.*, **47**, 2724 (1925).

[115] See: C. Scheibler and H. Mittelmeier, *Ber.*, **22**, 1680, 3120 (1889).

[116] C. Neuberg, *Biochem. Z.*, **3**, 528 (1907).

[117] W. N. Haworth, E. L. Hirst and associates, *J. Chem. Soc.*, **123**, 3125 (192); 1527, 3146 (1927)

[118] N Wattiez and M Hans, *Bull. Acad. roy. med. Belg.*, **8**, 386 (1943); *Chem. Abst.*, **39**, 4840 (1945).

Stachyose

α-Galactosyl $\overset{1\text{-}6'}{-}$ α-galactosyl $\overset{1'\text{-}4}{-}$ α-glucosyl - $\overset{1\text{-}2'}{-}$ - β-fructofuranoside
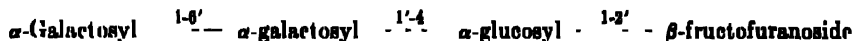
Synonyms. Lupeose, β-galactan, manneotetrose.

Properties. m.p., 167–170°C.; $[\alpha]_D^{20} = +148$. Partially fermentable by yeasts. Nonreducing.

Occurrence. The tetrasaccharide is a frequent constituent of plant products and is found associated with raffinose and sucrose. It has been reported in the roots of *Stachys* species, in the twigs of white jasmine, in the seeds of yellow lupine (*Lupinus luteus*), in soybeans (*Soja hispida*), in lentils (*Ervum lens*) and in ash manna (*Fraxinus ornus*).

Preparation.[119] Ash manna and nodules of *Stachys tuberifera* have been utilized as sources of the sugar.

Structure. The formula given is proposed by Onuki as a result of methylation studies. Since the sugar is often associated with raffinose, it seems peculiar that it is not derived from this sugar rather than an unknown isoraffinose with a 1.4' disaccharide connection between the galactose and glucose components.

Baker's yeast invertase preparations, which contain no α-galactosidase, hydrolyze stachyose to the trisaccharide manninotriose and fructose.[120] However, brewer's yeast emulsin, which contains invertase and α-galactosidase, completely hydrolyzes the sugar. Since there is no β-galactosidase in either material, the galactoside linkages must have the α-configuration.

A galactosylstachyose (verbascose) occurs in certain *Verbascum* (mullein) species. It has the galactosyl group substituted at carbon 6 of the terminal galactose unit of the stachyose.[121]

[119] C Tanret, *Bull. soc. chim.*, [3], *27*, 917 (1902), M Onuki, *Sci Papers Inst. Phys Chem Research (Tokyo)*, *20*, 201 (1933).

[120] M Adams, N K Richtmyer and C S Hudson, *J. Am. Chem. Soc.*, *65*, 1369 (1943)

[121] See S Murakami, *Proc Imp Acad (Tokyo)*, *16*, 12 (1940)

NATURALLY OCCURRING GLYCOSIDES AND GLYCOSIDASES

From a biological standpoint, the natural glycosides comprise one of the important groups of the carbohydrates. Bourquelot states that of 281 species of phanerogams investigated in his laboratories glycosides were found to be present in 205 species.[1] Many of the colored pigments of flowers, the naturally occurring dyestuffs and aromatic principles, and drugs such as the heart-stimulating (cardiac) glycosides are of glycosidic nature.

Numerous conjectures of the function of glycosides in plants have been made.[2] Glycosides may serve as reserve deposits for sugars, particularly in seeds. Other possible functions are as controls for osmotic pressure and for the stabilization of labile aglycons. By analogy with the use of glucosides, or particularly glucuronides, for detoxification by animals (see p. 509), it has been suggested that plant glycosides play a similar role by removing toxic materials or end products of metabolic processes. Plant glycosides are known to be localized in the cell vacuoles. Frey-Wyssling suggests that the aglycons are end-products of metabolic processes. The aglycons usually are lipophilic in character, but they also contain hydrophilic groups and are surface-active. These characteristics lead to the supposition that the aglycons will tend to be deposited along with the lipides in the surface of the border layer of the cytoplasm next to the vacuoles. Because their accumulation in this layer might be detrimental to the functions of the cell, it is likely that glycosidation occurs and the glycosides, now hydrophilic, pass into the aqueous phase of the vacuoles, which act as a disposal ground for the waste products of metabolism.

Although most common in plant materials, glycosides are also found in substances of animal origin; among the best known animal sources are brain tissue (cerebrosides, see under Galactose) and urine. Naturally occurring oligosaccharides, a type of glycoside, are discussed in a separate chapter (Chapter X). Because the principal interest in the glycosides resides in the chemistry of the aglycon (the nonsugar portion), the naturally occurring glycosides are considered in the present chapter along with the enzymes (glycosidases) which act as catalysts for their hydrolysis and synthesis and which often accompany the glycosides in natural products. General methods of synthesis and the definitions and properties of glycosides are considered in an earlier chapter (Chapter V). The so-called "nitrogen glycosides" (glycosylamines) are covered elsewhere (Chapter IX).

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[1] Quoted by C. Béguin, *Pharm. Acta Helv.*, *1*, 90 (1926).

[2] For a discussion of the subject see: A. Frey-Wyssling, *Naturwissenschaften*, *33*, 500 (1942).

GLYCOSIDES[a]

1. Anthocyanidin and Flavonol Glycosides

Many plant pigments occur as glycosides which on acid or enzymic hydrolysis yield a sugar, usually glucose, or a mixture of sugars, and the anthoxanthins which include the flavones, the flavonols, the flavanones, the isoflavones and the xanthones. The soluble red, violet and blue pigments of flowers, fruits and leaves which have been termed anthocyanins give a sugar and an anthocyani*din* (the aglycon) on hydrolysis. Before the widespread adoption of coal-tar base dyes, these substances had considerable value as dyestuffs. The subject is well reviewed, and the methods for the determination of the structures of these glycosides and their aglycons are given adequately elsewhere.[4] The synthesis of peonin (V), the anthocyanin pigment of the dark-red peony, is outlined below.[5]

Peonin   (V)
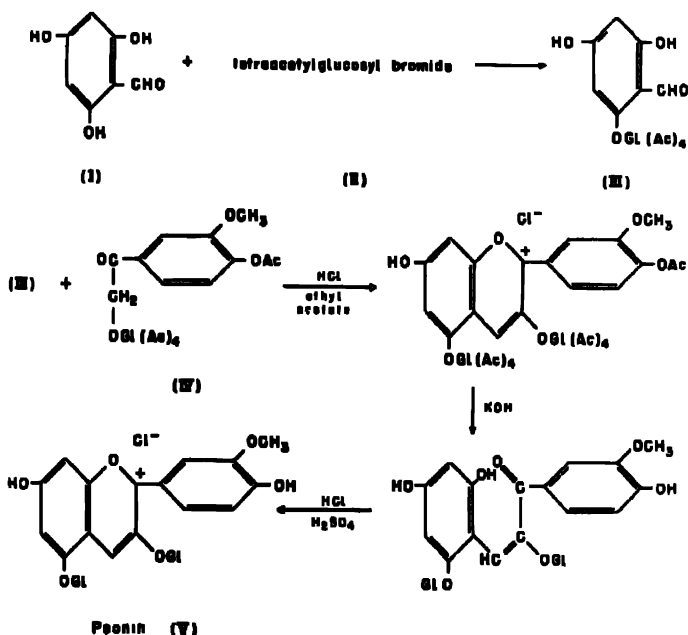
[Gl(Ac)₄ = tetraacetylglucosyl group; Gl = glucosyl group.]

[a] General references: E. F. and K. F. Armstrong, "The Glycosides;" Longmans, Green and Company, New York (1931); J. J. L. van Rijn and H Dieterle, "Die Glycoside;" Gebrüder Borntraeger, 2nd Ed., Berlin (1931); G. Klein, "Handbuch der Pflanzenanalyse;" J. Springer, Vienna (1932).

[4] K. P. Link, in "Organic Chemistry," p. 1315; Editor. H. Gilman, 2nd Ed., John Wiley and Sons, New York (1943); E. F. and K. F. Armstrong, "The Glycosides;" Longmans, Green and Company, New York (1931); R. Robinson, *Ber.*, 67A, 85 (1934)

[5] R. Robinson and A R. Todd, *J. Chem. Soc.*, 2488 (1932). For a discussion of the structure of flavylium chlorides of the type of V, see R. L. Shriner and R. B. Moffett, *J. Am. Chem. Soc.*, 63, 1694 (1941).

Quercitrin, a flavonol glycoside from the bark of certain oak trees is used for the preparation of L-rhamnose (see under this sugar). It has the structure:
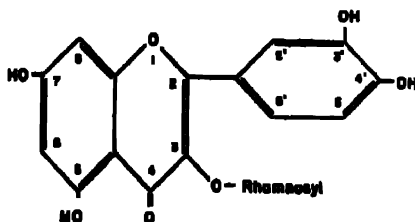
Quercitrin

The aglycon is known as quercitin. A number of natural glycosides yield quercitin derivatives on hydrolysis. Thus, xanthorhamnin obtained from the ripe fruit of *Rhamnus infectoria* yields one mole of rhamnetin, two moles of L-rhamnose and one of D-galactose. Rhamnetin is the 7-methyl ether of quercitin. Glycosides of the 3'-methyl ether of quercitin, isorhamnetin glycosides, also are naturally occurring. From crocus pollen, the 3,4'-diglucoside of isorhamnetin has been isolated.[6] Rhamnazin is the 3',7-dimethyl ether.

Some of the above and similar compounds exhibit a powerful influence on the sexual processes of the green alga *Chlamydomonas*. Thus the glucoside from crocus pollen (see above) in dilutions as low as 1 mg. in 6×10^9 ml. of water (about 80 molecules per cell) immobilizes the gametes of the alga, and the cilia drop off. The yellow aglycon acts on the bisexual cells and imparts to them the property of being able to conjugate only after the addition of male gametes.[7]

One of the flavanone glycosides, hesperidin, has been shown to be identical with vitamin P, which is concerned with the regulation of capillary permeability and fragility.[8] The glycoside is found in the peels of citrus fruits and on hydrolysis yields one mole each of glucose, rhamnose and the aglycon hesperitin. The sugar units in the hesperidin are united in disaccharide fashion to form 6-(β-L-rhamnosyl)-D-glucose (rutinose), and the hesperidin is hesperitin β-rutinoside.[9] The flavanone exists in equilibrium with its chalcone isomer, the chalcone being formed in alkaline solution and the hesperidin existing in acid solution.

[6] See: R. Kuhn and Associates, *Ber.*, **77**, 196, 202, 211 (1944).

[7] R. Kuhn, F. Moewus and I. Löw, *Ber.*, **77**, 219 (1944).

[8] C. Z. Wawra and J. L. Webb, *Science*, **96**, 302 (1942); A. Szent-Györgyi, *Z. physiol. Chem.*, **255**, 126 (1938).

[9] G. Zemplén and A. K. Tettamanti, *Ber.*, **71**, 2511 (1938); G. Zemplén and R. Bognár, *Ber.*, **76**, 773 (1943).

Hesperidin   Hesperidin chalcone
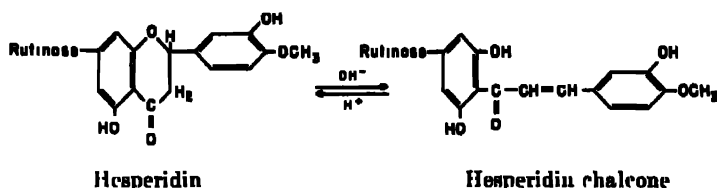
The chalcone takes up hydrogen readily and the reduced form loses hydrogen when shaken in air. The reduced chalcone also gives up hydrogen to the well known oxidation-reduction coenzymes (see Cozymase) and probably plays such a role in biological systems.

The hesperidin frequently is accompanied by another glycoside, eriodictyol glycoside. This substance has been shown to be closely related to hesperidin. The methoxyl group (see above formula) is replaced by a hydroxyl group, and the sugar component is L-rhamnose rather than rutinose.[10]

The glycoside rutin, 3-(3,5,7,3',4'-pentahydroxyflavone) rutinoside, causes a decrease in capillary fragility in a manner resembling hesperidin.[11]

2. Indican

In addition to the anthocyanins, another important source of dyes is the naturally occurring indican which on hydrolysis yields indoxyl and glucose. The indoxyl upon oxidation is converted to the dye indigo. The preparation of indigo from plants involves the extraction of the glucoside, its enzymic hydrolysis by microorganisms and the oxidation of the indoxyl to indigo by air. The synthesis by Robertson[12] of indican, illustrated below, furnishes
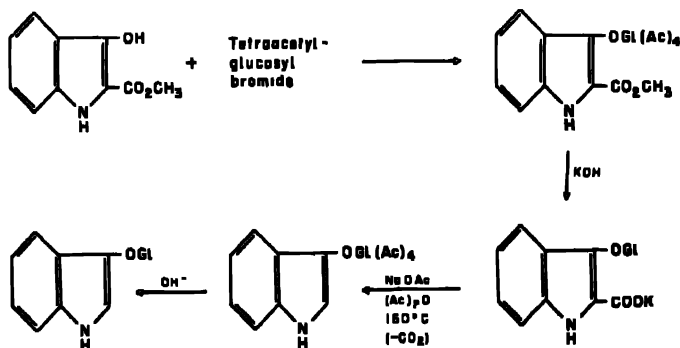
Indican

(Gl(Ac)₄ = tetraacetylglucosyl group; Gl = glucosyl group.)

[10] A. Mager, Z. physiol. Chem., 274, 109 (1942).

[11] J. Q. Griffith, Jr., J. F. Couch and M. A. Lindauer, Proc. Soc. Exptl. Biol. Med., 55, 228 (1944).

[12] A. Robertson, J. Chem. Soc., 1937 (1927).

the final evidence needed for the structural determination and demonstrates the glycoside to be 3-hydroxyindole β-D-glucoside.

Normal metabolism of tryptophan in animals leads to the production of indoxyl glucuronides (and sulfate) in the urine.[2]

3. Aglycons Related to Phenanthrene

A. Cardiac glycosides.[13] These glycosides are of considerable medical interest because of their stimulatory action on the heart. Some of the many plant families in which the presence of these glycosides has been demonstrated are: Liliaceae, Ranunculaceae, Scrophulariaceae and Apocynaceae. The most important sources are certain species of *Strophanthus* and *Digitalis* (foxglove), the latter providing most of the drugs of therapeutic value. Many if not all of the cardiac glycosides have a desoxysugar (rhamnose, digitoxose or cymarose) as one of the component sugars and usually this sugar is attached directly to the aglycon. The general formula of a cardiac glycoside may be expressed as follows:[14]

$$\text{Aglycon-(desoxy sugar)}_m\text{-(glucose)}_n$$

Acids hydrolyze the linkage between the aglycon and the sugars in the cardiac glycosides, and di- and tri-saccharides can be isolated. It is stated that this linkage is not hydrolyzed by the enzymes in the same plant, but instead only the glycosidic bonds between the sugars are split. Many of the

O-Rhamnose-β-glucose   O Digitoxose digitoxose digitoxose-β-glucose

Proscillaridin *A*   Scillabiose   Acetyl-digoxin

Scillaren *A*,   Lanata-glycoside *C*,

Principal glycoside   Glycoside from *Digitalis lanata*

of *Scilla maritima*
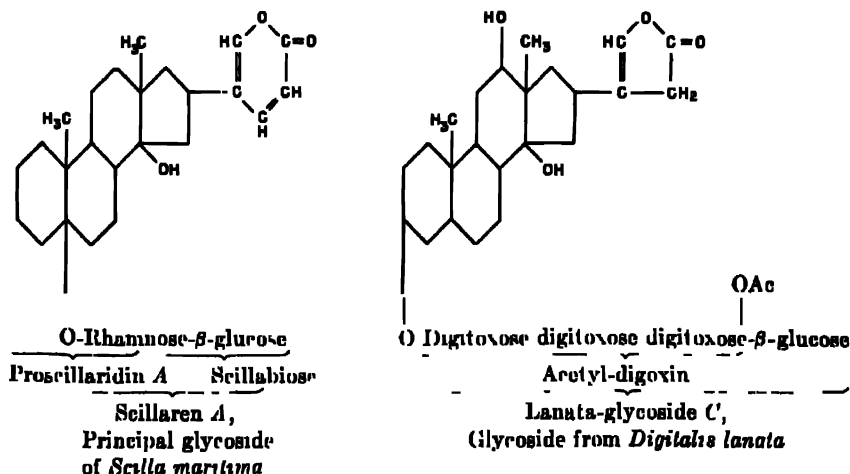
[13] R. C. Elderfield, *Chem. Revs.*, **17**, 187 (1935); A. Stoll, "The Cardiac Glycosides," Pharm. Press, London (1937); W. H Strain in "Organic Chemistry," p. 1427; Editor: H. Gilman, 2nd ed , John Wiley & Sons, New York (1943); L F. Fieser, "The Chemistry of Natural Products Related to Phenanthrene," p. 256, A C. S. Monograph 70, Reinhold Publishing Corporation, New York (1937).

[14] A. Stoll and J. Renz, *Enzymologia*, **7**, 362 (1939).

products which have been obtained from the above-mentioned plants are undoubtedly partially degraded due to the loss of glucose by enzymic action during the process of preparation. The structures of several typical cardiac glycosides and of the degradation products are given in the accompanying formulas. It will be noted that the aglycons are lactones all very similar in structure. The aglycon ring structures are similar to those of the sterols, vitamin D, sex hormones, bile acids, neutral saponins and certain adrenal substances, all being derived from the hydrocarbon cyclopentanoperhydrophenanthrene.

When administered to persons with impaired heart function, cardiac glycosides produce an increased intensity of heart beat and a decreased rate. Some of these glycosides have been used as arrow poisons.

O-Cymarose-β-glucose-α-glucose

Strophanthobiose

Cymarin   Strophanthotriose

k-Strophanthin-β

k-Strophanthoside from *Strophanthus kombé*
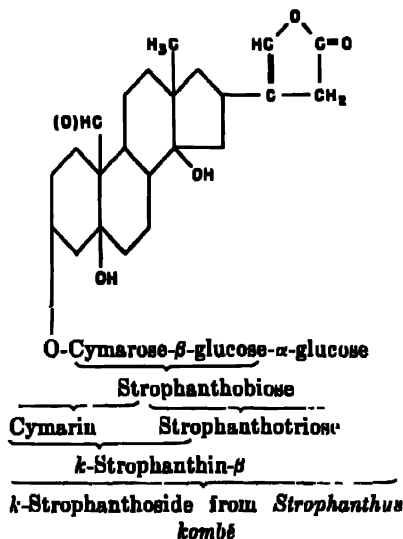
The influence of the nature of the glycosidic group of the strophanthidin glycosides on their biological activity is illustrated in Table I. The mean lethal dose (cat assay) and the mean systolic dose (frog assay) for a number of natural and synthetic glycosides are compared with those for the unsubstituted aglycon (strophanthidin).[15] It is of interest that the glucoside and L-arabinoside are more active than the natural product (the cymaroside).

There is some evidence that the true hormone of the adrenal cortex is a glycoside, and several synthetic glycosides of this type have been prepared.[16] The desoxycorticosterone glucoside is more soluble in water than the

[15] F. C. Uhle and R. C. Elderfield, *J. Org. Chem.*, **8**, 162 (1943).
[16] W. S. Johnson, *J. Am. Chem. Soc.*, **63**, 3238 (1941).

aglycons and exhibits full physiological activity in maintaining the life of adrenalectomized rats.[17] Synthetic glucosides of cholestanol and epicholestanol[18] and the methyl esters of several sterol galacturonides[19] are reported.

The Solanum alkaloids, occurring in species of *Solanum*, including potatoes, "Dead Sea Apple" and "poro-poro," also contain the sterol nucleus and exist as glycosides.[20] On hydrolysis, a nitrogen base and equimolecular quantities of L-rhamnose, D-galactose and D-glucose are produced. Partial hydrolysis produces first rhamnose, then galactose and finally glucose. Solanine, from potato sprouts, is given the following abbreviated formula:

$$C_{27}H_{42}N-O-glucose-galactose-rhamnose$$

TABLE I

Biological Activity of Strophanthidin Glycosides

Substance	Mean Lethal Dose (cat assay) (micrograms/kg.)	Mean Systolic Dose (frog assay) (micrograms/kg.)
Strophanthidin	306.2	2 71
Strophanthidin β-glucoside	91 3	0 583
Strophanthidin β-glucoside tetraacetate	1106	18.77
Strophanthidin β-xyloside	100.5	0.64
Strophanthidin β-xyloside triacetate	591.6	8.07
Strophanthidin L-arabinoside	94.5	0.308
Strophanthidin L-arabinoside triacetate	1230	6.33
Strophanthidin β-galactoside tetraacetate	1692	11.20
Strophanthidin cymaroside (cymarin)	110.1	0.60

The same trisaccharide seems to be unique to the *Solanum* species, and is found in the other *Solanum* alkaloids. It is probably connected by a glycosidic linkage to a hydroxyl at carbon 3 of the sterol nucleus.

B. Saponins.[21] The saponins are an important class of glycosides widely distributed in plants. Saponin solutions foam easily. Given intravenously they are poisonous and possess hemolytic action. They are non-toxic, as a rule, when administered orally, probably because of lack of absorption from

[17] K. Miescher, W. H. Fischer and Ch. Meystre, *Helv. Chim. Acta*, **25**, 40 (1942).

[18] R. P. Linstead, *J. Am. Chem. Soc.*, **62**, 1766 (1940).

[19] H. Sell and K. P. Link, *J. Biol. Chem.*, **125**, 235 (1938).

[20] G. Oddo and G. Caronna, *Ber.*, **67**, 446 (1934); L. H Briggs, R. Newbold and N. E. Stace, *J. Chem. Soc.*, 3 (1942); L. H. Briggs and J. J. Carroll, *ibid.*, 17 (1942).

[21] W. H. Strain, *in* "Organic Chemistry," p. 1454; Editor: H. Gilman, 2nd ed., John Wiley and Sons, New York (1943); L. Kofler, "Die Saponine," J. Springer, Vienna (1927); R. Tschesche, *Ergeb. Physiol.*, **33**, 65 (1936); L. F. Fieser, "The Chemistry of Natural Products Related to Phenanthrene," p 317; A. C. S. Monograph 70, Reinhold Publishing Corporation, New York (1937).

the intestine. Their use as fish poisons depends on this fact, and fish which have been killed by the addition of plant extracts containing saponins to fish-bearing waters may be safely consumed by humans.

Two classes are recognized, the so-called neutral saponins (digitalis saponins), which have as aglycons substances derived from cyclopentano-perhydrophenanthrene, and the acid saponins, which have as aglycons substances related to 1,2,7-trimethylnaphthalene. The aglycons are called sapogenins. Most of the investigations have been devoted to the determination of the structures of the neutral saponins.

The provisional formula[24] of a typical neutral saponin is that illustrated for sarsasaponin (parillin) from *Radix sarsaparillae*.

Sarsasaponin

(Glucose, glucose, rhamnose)
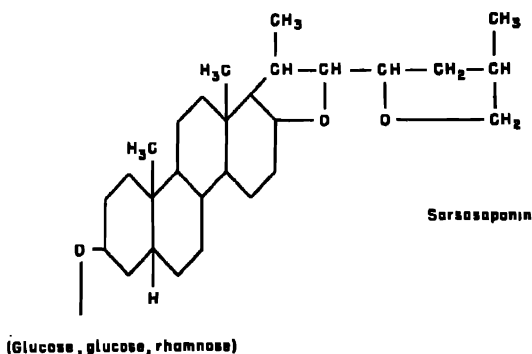
4. Substituted-Phenyl Glycosides

Many substituted phenols are found as the aglycons of naturally occurring glycosides. Arbutin (hydroquinone β-D-glucoside) and methylarbutin (p-methoxyphenyl β-D-glucoside) are extracted from the leaves of the bearberry (*Arctostaphylos uva-ursi*) and frequently occur together in other plants particularly those of the family Ericaceae. The substances have been synthesized by the reaction between the corresponding salt of the phenol and tetraacetylglucosyl halide.[25] The leaves of certain varieties of *Pyrus* (pear family) turn black when they fall, but others assume an intermediate yellow color. These differences are believed to be due to variations in the arbutin and methylarbutin content. Leaves with considerable amounts of arbutin form hydroquinone by enzymic hydrolysis, and the hydroquinone is then oxidized by the air directly to a black product. The hydroquinone methyl ether obtained from the methylarbutin oxidizes first to a transient yellow substance before the black color develops. Arbutin has been employed in medicine for its internal antiseptic effect and its diuretic action.

[24] J. C. E. Simpson and W. A. Jacobs, *J. Biol. Chem*, *109*, 573 (1935); S. N. Farmer and G. Kon, *J. Chem. Soc.*, 414 (1937).

[25] A. Michael, *Ber*, *14*, 2097 (1881); C. Mannich, *Arch. Pharm.*, *250*, 547 (1912).

Salicin is a glucoside found in willow bark (*Salix*) and in poplar bark (*Populus*). Particularly in the poplar bark, it is found accompanied by a second glucoside, populin. Salicin is *o*-hydroxymethylphenyl β-D-glucoside, while populin is the 6-benzoyl derivative.[24] The salicin is hydrolyzed easily by enzymes in almond emulsin, but populin is unaffected.[25] However, it is claimed that an enzyme present in *Populus monilifera* hydrolyzes populin to salicin and benzoic acid.[26] These substances have had some medicinal application as remedies for fever and acute rheumatism.

5. Vanillin and Coumarin Glucosides

Vanillin, the aromatic principle of vanilla extract, occurs in many plants as the glycoside and, in particular, in the vanilla bean (*Vanilla planifolia* Andrews) of commerce. The curing of the beans is essentially an enzymic hydrolysis with the formation of the free vanillin. The glucoside and related glucosides have been synthesized,[27] and the vanillin β-D-glucoside is the most easily hydrolyzed of all the glycosides cleaved by almond emulsin. An old but now little-used method for the production of vanillin depends on the oxidation of the glucoside, coniferin, which occurs in the sap of fir trees.

Coniferin   Vanillin
β glucoside
(Glucovanillin)

    Vanillin
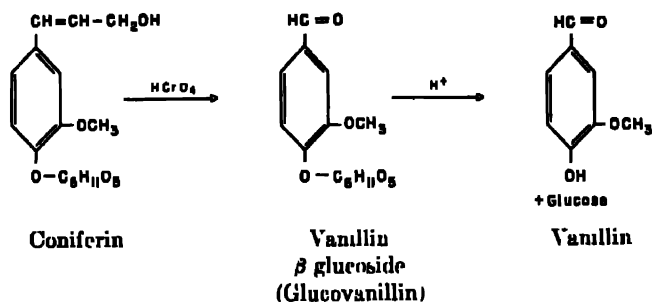
It is usually considered that the aromatic principle coumarin which is found in many plants occurs as the glucoside. Since it has no free hydroxyl, it is probably present in the plant as the glucoside of *o*-coumaric acid which isomerizes into the *cis* form, coumarinic acid, and then to the lactone coumarin. The principal natural source of the coumarin is the tonka bean (*Dipteryx oppositifolia* Willd.).

[24] P. Piria, *Ann.*, *56*, 35 (1845); N. K. Richtmyer and E. Yeakel, *J. Am. Chem. Soc.*, *56*, 2495 (1934).

[25] W. W. Pigman and N. K. Richtmyer, *J. Am. Chem. Soc.*, *64*, 374 (1942).

[26] T. Woevers, *Koninkl. Akad. Wetenschappen Amsterdam*, *12*, 193 (1909).

[27] See: B. Helferich, H. E. Scheiber, R. Streeck and F. Vorsatz, *Ann.*, *518*, 211 (1935).

| o-Coumaric acid glucoside | o-Coumaric acid (*trans* isomer) | Coumarinic acid (*cis* isomer) | Coumarin |

The glucoside of the *o*-coumaric acid (melilotin) has been isolated[28] from flowers of *Melilotus altissima* and has also been synthesized.[29] Glycosides of hydroxyl- and methoxyl-substituted coumarin are also widely distributed [30]

6. Cyanogenetic Glycosides

One of the earliest known glycosides is amygdalin, the effective principle of "oil of bitter almonds." Most plants belonging to Rosaceae (apricot,

Heptaacetylgentiobiosyl bromide

Amygdalin

[28] C. Charaux, *Bull. soc. chim. biol.*, 7, 1056 (1925).

[29] B. Helferich and H. Lutzmann, *Ann.*, 537, 11 (1939).

[30] E. F. and K. F. Armstrong, "The Glycosides," p. 19; Longmans, Green Company, New York (1931).

bitter almond, plum, peach, etc.) contain considerable quantities of the glycoside or other cyanogenetic glycosides in the kernels, leaves and woody portions. Acid or enzymic hydrolysis converts amygdalin to one mole each of benzaldehyde and hydrogen cyanide and two moles of glucose. The enzymic hydrolysis of amygdalin is of particular interest because it was one of the earliest-observed instances of enzymic action.[31] The responsible substance was named "emulsin," a term now suggested for mixtures of enzymes (see p. 475).

Since the amygdalin may be synthesized[32] from heptaacetylgentiobiosyl bromide and ethyl D,L-mandelate by the procedure outlined (p. 468), the glycoside is undoubtedly D(levo)-mandelonitrile β-D-gentiobioside.[33]

The enzymic hydrolysis by almond emulsin (β-glucosidase component) requires the preliminary hydrolysis of the gentiobiose into glucose and mandelonitrile β-D-glucoside before the aglycon group is removed,[34] but the hydrolysis by other enzymes may follow a different course.

It is claimed that yeast extracts will hydrolyze amygdalin to mandelonitrile β-glucoside which is identical with the glucoside prunasin found in wild cherry bark (*Prunus serotina*). A glucoside isomeric with prunasin and called sambunigrin is found in elder leaves (*Sambucus niger*). These glucosides differ only in the nature of the aglycon group; that for prunasin is D(levo)-mandelonitrile β-glucoside whereas that for sambunigrin is L(dextro)-mandelonitrile β-glucoside. In alkaline solution these two isomeric glucosides are racemized to a mixture of the two which is called prulaurasin.[35] The synthesis of these substances from the synthetic ethyl mandelates and tetraacetylglucosyl bromide has been accomplished.[36]

7. Hydroxyanthraquinone Glycosides

These important substances are found in many plant materials, and the aglycons have had considerable value as dyestuffs. Ruberythric acid, the principal constituent of madder (the ground root of *Rubia tinctoria*), is hydrolyzed by the enzymes of *Primula officinalis* and *P. vulgaris* emulsin to alizarin (1,2-dihydroxyanthraquinone) and primeverose (6-D-glucose β-xyloside). Since methylation of the ruberythric acid and acid hydrolysis yields alizarin 1-methyl ether, the glycoside is 2-alizarin β-D-primeveroside.[37] This structure has received a final proof in the synthesis of the glycoside from alizarin and hexaacetylprimeverosyl bromide in an aqueous

[31] F. Wöhler and J. Liebig, *Ann.*, 22, 1 (1837).
[32] R. Campbell and W. N. Haworth, *J. Chem. Soc.*, 1337 (1924); G. Zemplén and A. Kunz, *Ber.*, 57, 1357 (1924); R. Kuhn and H. Sobotka, *ibid.*, 57, 1767 (1924).
[33] For the configuration of mandelic acid see: K. Freudenberg *et al.*, *Ber.*, 56, 193 (1923).
[34] R. Weidenhagen, *Ergeb. Enzymforsch.*, 1, 197 (1932).
[35] See: R. J. Caldwell and S. L. Courtauld, *J. Chem. Soc.*, 91, 666, 671 (1907).
[36] E. Fischer and M. Bergmann, *Ber.*, 50, 1047 (1917).
[37] D. Richter, *J. Chem. Soc.*, 1701 (1936).

acetone-KOH solution.[38] Other glycosides present in madder are: purpurin (1,2,4-trihydroxyanthraquinone D-glucoside) and rubiadin glucoside[39] (3-(1,3-dihydroxy-2-methylanthraquinone) D-glucoside). The presence of these glucosides along with the ruberythric acid in madder is the probable explanation for the well-known color difference between the natural and the synthetic alizarin.

8. Sugar Components of Natural Plant Glycosides

The most frequently encountered sugar component of glycosides is D-glucose, but practically all of the naturally occurring sugars are found in plant glycosides. Peculiarly enough, three of the most common sugars (D-galactose, D-mannose and D-fructose) are only rarely encountered. Galactose is reported to occur in certain saponins and trisaccharide glycosides (robinose and rhamninose). A mannoside is found in some sea weeds. Fructose is reported to be the sugar of certain saponins. In contrast, the 6-desoxy-L-mannosides and 6-desoxy-D and L-galactosides (L-rhamnosides and D-and L-fucosides) are often found in plant products. Of the pentoses, L-arabinose and D-xylose are frequent and D-arabinose infrequent constituents of glycosides. Glucuronides also are reported.

In addition to the sugars mentioned, a group of desoxysugars and branched-chain sugars is found as constituents of the digitalis glycosides. Digitoxose is 2,6-didesoxy-D-allose.[40] The designation of digitalose as a 2-methyl-6-desoxyhexose has been questioned, and it is more probably 3-methyl-D-fucose.[41] Similar rare sugars, cymarose and sarmentose obtained by the hydrolysis of certain strophanthidin glycosides, are believed[42] to be 2,6-didesoxy-3-methylhexoses. Cymarose has been further identified[43] as 2,6-didesoxy-3-methylallose (3-methyldigitoxose).

Digitoxose	Digitalose	Cymarose
CHO	CHO	CHO
HCH	HCOH	HCH
HCOH	CH₃OCH	HCOCH₃
HCOH	HOCH	HCOH
HCOH	HCOH	HCOH
CH₃	CH₃	CH₃
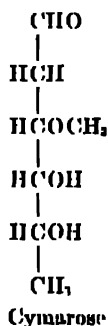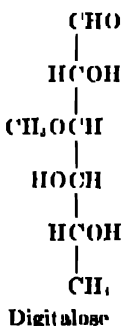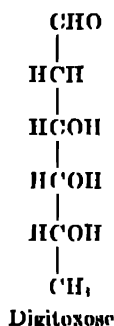
[38] G. Zemplén and R. Bognár, Ber., 72, 913 (1939).

[39] E. T. Jones and A. Robertson, J. Chem. Soc., 1699 (1930).

[40] F. Micheel, Ber., 63, 347 (1930); B. Iselin and T. Reichstein, Helv. Chim. Acta, 27, 1203 (1944).

[41] F. G. Young, Jr., and R. C. Elderfield, J. Org. Chem., 7, 241 (1942); O. Th. Schmidt and E. Wernicke, Ann., 556, 179 (1944).

[42] W. A. Jacobs and N. M. Bigelow, J. Biol. Chem., 96, 355 (1932).

[43] R. C. Elderfield, J. Biol. Chem., 111, 527 (1935).

What is apparently the enantimorph of oleandrose has been synthesized from 3-methyl-D-glucose.[44] Hence, oleandrose appears to be a 2-desoxy-3-methyl-L-rhamnose (= 2,6-didesoxy-3-methyl-L-glucose). This sugar and diginose (of unknown structure) have been isolated from cardiac glycosides.

A sugar interesting because of its unusual branched-chain structure is apiose, which occurs conjugated with the 5,7,4'-trihydroxyflavone as the glycoside apiin in the leaves and seed of parsley. The only other branched-chain sugars known are hamamelose and a component of streptomycin. The structure of apiose is shown by the reduction of apionic acid with phosphorus and hydrogen iodide to 3-methylbutyric acid.[45] The single asymmetric carbon of apiose probably has the configuration of D-(levo)-lactic acid.

$$\begin{array}{c} \text{HOCH}_2 \\ | \\ \text{C(OH)—CH(OH)--CHO} \quad \xrightarrow{\text{Ba(IO)}_3} \quad \xrightarrow[\text{P}]{\text{HI}} \quad (\text{CH}_3)_2\text{CH—CH}_2\text{—COOH} \\ | \\ \text{HOCH}_2 \end{array}$$

Apiose
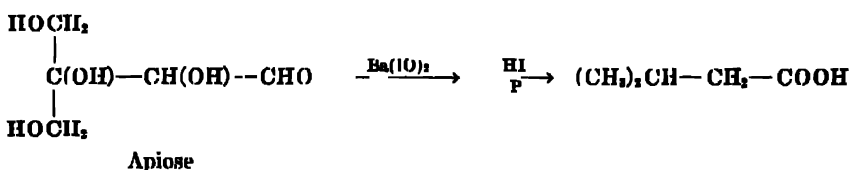
In the plant materials from which the glycosides are obtained by extraction, the corresponding enzymes are often to be found. If care is not taken to destroy the enzyme, hydrolysis of the glycoside may take place and erroneous conclusions be drawn concerning the sugars present. Hydrolysis is prevented by rapid heating of the aqueous or alcoholic solutions to the boiling temperature. Because this precaution has not always been taken, many reported monosaccharide constituents may really be disaccharides or trisaccharides. In many instances it is known that the sugar portion of the glycoside is a di- or trisaccharide. In other instances an oligosaccharide structure is assumed if several molecules of a sugar are formed from a single mole of the glycoside although, if the aglycon is a polyhydroxyphenol, the glycoside may be a di- or triglycoside. The structures of some di- and trisaccharides found as constituents of glycosides are described in the chapter on oligosaccharides.

The naturally occurring glucosides have almost exclusively the beta configuration for the glucosidic carbon and as a result are levorotatory. Dextrorotating phillyrin from *Forsythia suspensa* and *Olea fragrans* is believed[46] to be an α-D-glucoside, for it is hydrolyzed by yeast α-glucosidase and not by almond emulsin. Alkyl glycosides are rare, but the ethyl α-D-galactoside has been obtained[47] by the extraction of the phosphatides of yellow sweet lupines; floridoside, a crystalline glycoside found in red algae (*Florideae*), is considered to be 2-glycerol α-D-galactoside.[48] Methyl β-D-

[44] E. Vischer and T. Reichstein, *Helv. Chim. Acta*, **27**, 1332 (1944).

[45] O. Th. Schmidt, *Ann.*, **483**, 115 (1930).

[46] F. Kollo and T. Hjerlow, *Pharm. Zentr.*, **71**, 705 (1930).

[47] E. Nottbohm and F. Mayer, *Vorratspflege u. Lebensmittelforsch.*, **1**, 243 (1938).

[48] H. Colin, *Bull. soc. chim.*, [5] **4**, 277 (1937).

glucoside has been isolated from the fresh leaves of *Scabiosa succisa*.[49] A D-glyceric acid α-mannoside occurs in algae of the genus *Polysiphonia*.[50]

9. Thioglycosides and thiosugars[51]

Black mustard (*Brassica nigra* Koch) contains a glucoside, sinigrin, which is hydrolyzed by enzymes present in the plant to allyl isothiocyanate, potassium hydrogen sulfate and glucose. The presence of this glucoside explains the odor of allyl isothiocyanate which develops when the seed is bruised and moistened. According to J. Gadamer, the structure is:

$$OSO_3K$$

$$CH = CH \quad CH_2 \quad N = C - S \quad (C_6H_{11}O_5)$$

Almond emulsin does not hydrolyze the glucoside, but mustard seed emulsin contains an enzyme, myrosin, which catalyzes the hydrolysis. It seems probable that the linkage is beta and indeed enzymic hydrolysis produces β-glucose. However, silver nitrate and silver carbonate produce α-glucose, presumably as a result of a Walden inversion.[52] The hydrolytic action of sodium hydroxide leads to thioses (1-thiosugars)

Many of the plants of *Cruciferae* produce sinigrin and other sulfur containing glycosides. These compounds comprise the so-called mustard-oil glycosides and are described in more detail elsewhere.[51]

Synthetic thioglucosides are prepared by the action of thiophenol on acetylglycosyl bromides in the presence of sodium hydroxide.[53,54] They are extremely resistant to acid hydrolysis, and this resistance probably explains the lack of hydrolysis by almond emulsin.[54,55] By treatment of sugar mercaptals with mercuric chloride, thioglycosides also may be synthesized (see p. 195).

$$\begin{array}{ccc}
HC(SC_2H_5) & & HC \quad SC_2H_5 \\
| & \xrightarrow[H_2O]{HgCl_2} & | \\
HCOH & & HCOH \quad O \\
& & |
\end{array}$$
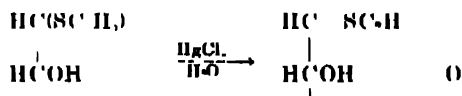
Many aromatic β-thioglucosides have been tested as antimalarials. Some

[49] L. N. Wattiez, *Chem. Abst.*, *19*, 3284 (1925).

[50] H. Colin and J. Augier, *Compt. rend.*, *208*, 1450 (1939).

[51] For reviews see: J. Gadamer, *Arch. Pharm.*, *235*, 44 (1897); H. Will and W. Körner, *Ann.*, *125*, 257 (1863); A. L. Raymond *Advances in Carbohydrate Chem.*, *1* 129 (1945).

[52] W. Schneider, H. Fischer and W. Specht, *Ber.*, *63*, 2787 (1930).

[53] E. Fischer and K. Delbrück, *Ber.*, *42*, 1476 (1909); C. B. Purves, *J. Am. Chem. Soc.*, *51*, 3627 (1929).

[54] W. Schneider, J. Sepp and O. Stiehler, *Ber.*, *51*, 220 (1918).

[55] W. W. Pigman, *J. Research Natl. Bur. Standards*, *26*, 197 (1941).

show a slight positive action, but the effect is too small to be of value.[55]

The sulfur analogs of the sugars are known as thiosugars.[57] When the sulfur replaces the oxygen of the anomeric hydroxyl (hydroxyl of carbon 1 of the aldoses), the compounds are sometimes distinguished as thioses, e.g., glucothiose and cellobiothiose. In addition to the thioglucosides discussed above, the only naturally occurring derivative of a thiosugar is the 5-thio-methylribose reported to occur, combined with adenosine, in yeast extracts (see under Nucleosides).

The 1-thioglucose is obtained by the alkaline hydrolysis of thioglucosides, and glucose 1-thiourethanes, 1-xanthates and 1-thio esters.[57,58]
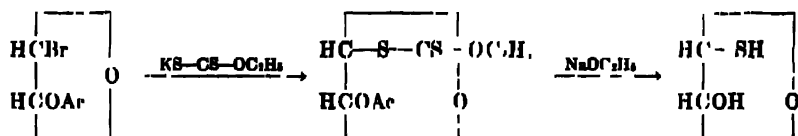
A 3-thioglucose has also been synthesized from the 3-glucose xanthate.[59] R - S - groups may be introduced into sugars by the addition of mercaptans to epoxy derivatives (see Inner Ethers). By analogy with the well known interconversion of cystein and cystine, disulfide derivatives are readily formed from the thioses and are the usual products obtained by the alkaline hydrolysis of thioglucosides. The following series of interconversions have been carried out for the xylose derivatives.[60]

Tetraacetylxylosyl bromide

Dixylosyl disulfide
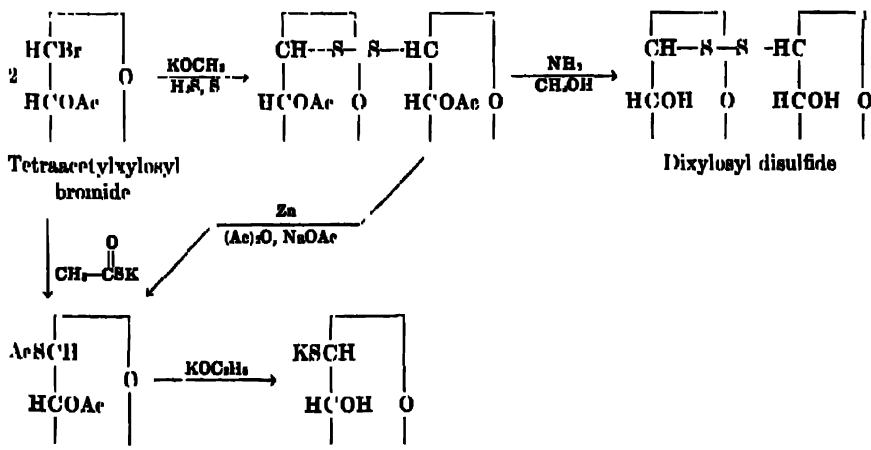
[55] E. M. Montgomery, N. K. Richtmyer and C. S. Hudson, *J Org. Chem.*, *11*, 301 (1946).

[57] W. Schneider, R. Gille and K Eisfeld, *Ber.*, *61*, 1244 (1928).

[58] F. Wrede. *Ber.*, *52*, 1756 (1919); E. Schwenk and M. Gehrke, U. S. Patent 2,088,-009, April 28, 1936.

[59] K. Freudenberg and A. Wolf, *Ber.*, *60*, 232 (1927).

[60] M. Gehrke and W. Kohler, *Ber.*, *64*, 2696 (1931).

Reduction of octaacetyldiglucosyl disulfide using Raney nickel gives tetraacetylpolygalitol (1,5-anhydrosorbitol).

The tosyloxy groups esterified with primary hydroxyls can be replaced by thiocyanate groups, and the resulting esters converted to disulfides in which two sugar molecules are connected by a disulfide linkage between the primary alcoholic groups.[61]

10. Streptomycin[61a]

Streptomycin, an important antibiotic, was first prepared as a crude concentrate by Waksman and coworkers from cultures of *Streptomyces griseus*, a soil organism. It is a glycoside of a unique type: a substituted *i*-inositol α-glycoside. The *i*-inositol carried two guanido ($NH_2 \cdot C$: NH·NH-) groups, and the monosaccharide units of the disaccharide are N-methyl-L-glucosamine and a branched-chain hexose with two aldehyde groups. The formula is given below; the configuration of the branched-chain hexose has not been defined completely, but appears to be that of L-ribose or L-lyxose.

R = *i* 3,5 diguanido-
2,4 6-trihydroxycyclohexyl

Streptomycin
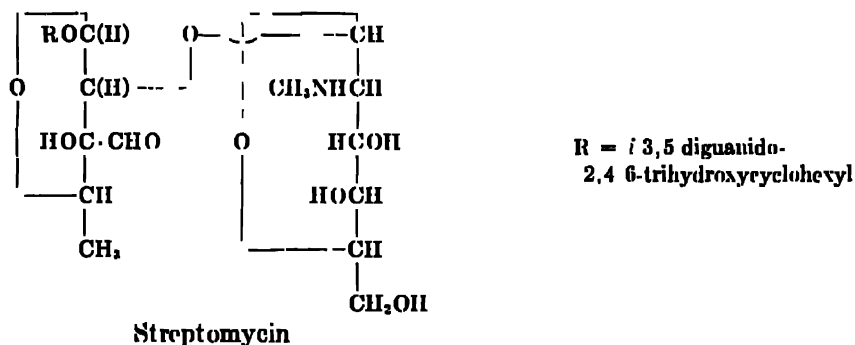
GLYCOSIDASES[62]

1. Introduction and Classification

Many materials of biological origin contain enzymes capable of catalyzing the hydrolysis of naturally occurring glycosides and oligosaccharides. These

[61] A. Müller and A Wilhelms, *Ber.*, **74**, 698 (1941).

[61a] For a review of the subject and detailed references see R. U. Lemieux and M. L. Wolfrom, *Advances in Carbohydrate Chemistry*, **3**, 337 (1947).

[62] A considerable amount of the material in this part has appeared elsewhere: W W. Pigman, *Advances in Enzymology*, **4**, 41 (1944).

enzymes usually are associated with their substrates, and to obtain the latter it is necessary to destroy the enzymes before hydrolysis takes place. Enzymes which hydrolyze glycosides and oligosaccharides are known as glycosidases; those which hydrolyze polysaccharides are known as polysaccharidases. As is also true for the substrates, no sharp distinction can be drawn between the glycosidases and the polysaccharidases and it is possible that each group of enzymes may exhibit some but usually slight action on the substrates of the other group. Thus, β-fructofuranosidase (invertase) may hydrolyze inulin as well as its natural substrate sucrose since both compounds have β-fructofuranosidic linkages. The glycosidases and polysaccharidases comprise the carbohydrases. Polysaccharidases are discussed under the corresponding polysaccharides. (See under Starch, Cellulose, etc.)

From the historical standpoint the glycosidases are of particular interest since they were the first enzymes to be known. Planche (1810, 1820) showed that the extracts of certain plants will cause guaiacum tincture to become blue. It was then demonstrated that extracts of the bitter almond hydrolyze the glycoside amygdalin also found in the bitter almond (Robiquet and Boutron-Chalard, 1830). The active principle of the bitter almond was further investigated and named emulsin (Liebig and Wöhler, 1837; Robiquet, 1838). Although a diastase in germinated barley was described in 1815 by Kirchoff, salivary amylase by Leuchs in 1831, and other cereal diastases in 1833 (Payen and Persoz), the first of the proteases (pepsin) was not reported until 1836 by Schwann.

The glycosidases are not of great industrial importance at present although the polysaccharidases are of the utmost interest from this standpoint. The amylases play important roles in the fermentation and baking industries and are of considerable biological importance. Other polysaccharidases such as cellulases and inulases are of considerable potential importance, for the corresponding polysaccharides are widely distributed. Of the glycosidases, β-fructofuranosidase or invertase, which hydrolyzes sucrose, is the most important although α-glucosidase (maltase) should be of considerable interest because of the common occurrence of maltose in commercial products. Most of the research carried out with these enzymes has centered around the amylases, the β-glucosidase and other enzymes of almond emulsin, and yeast invertase. The glycosidases provide excellent enzymes for specificity studies since the number of substrates which may be synthesized is practically limitless and since the compounds are well defined, easily prepared and usually crystalline. Although the glycosidases offer these advantages to the investigator, studies in this field suffer from the lack of purity of the enzymes and particularly in not having crystalline enzymes.

The historical name "emulsin" as applied to the active principle of the preparation from almonds has gradually assumed the meaning of a crude

mixture of glycosidases from any source. Helferich and Vorsatz[61] have used the term "emulsin" in this sense, but the definition has been broadened to include other enzymes. It is suggested that the partially purified enzyme mixtures obtained from seeds, microorganisms and animal organs and tissues be termed emulsins. Commercial "enzymes" are known as emulsins according to this definition. Almond emulsin is a mixture of enzymes prepared from almonds and not the β-glucosidase therein. Commercial invertase is a yeast emulsin and Takadiastase is an *Aspergillus oryzae* emulsin.

The individual glycosidases of the emulsins are named according to the α- or β-hexoside which they hydrolyze, as α- or β-hexosidases. Thus, β-glucosidases (earlier emulsin or prunasin) catalyze the cleavage of β-glucosides, and α-glucosidases the cleavage of α-glucosides.

A provisional classification[61] of the glycosidases is presented in Table II which lists the known types of carbohydrases and their substrates. The basis for this classification is given elsewhere although some evidence is given later in this chapter.

2. Mechanism of Action

The most widely accepted theory of enzyme action is based on the formation of an intermediate compound or adsorption complex between enzyme and substrate (Brown, 1902; Henri, 1903). Since both conceptions of the nature of the enzyme-substrate complex can lead to the same kinetic equations, the distinction seems unimportant at present. In the following development of the kinetic equations, the original scheme of Michaelis and Menten (1913) will be followed and compound formation will be considered to take place. However, in later discussions, the process will be considered as a type of adsorption.

A. Kinetic Equations and Effect of Substrate Concentration. In order to develop the kinetic equations, consider the hydrolysis of a glucoside (S) by an enzyme (E) to an alcohol or phenol (ROH) and glucose. The reactions may be represented:

$$S + E \leftrightarrows ES \tag{1}$$

$$ES + H_2O \to E + ROH + \text{Glucose} \tag{2}$$

Since a certain portion of both enzyme and substrate always is combined, the concentration of free enzyme $[E]$ and substrate $[S]$ at any time is given by the following equations, where the total enzyme concentration is represented by "c" and the total substrate concentration by "A."

$$[S] = [A] - [ES] \tag{3}$$

$$[E] = c - [ES] \tag{4}$$

[61] B Helferich and F. Vorsatz, *Z. physiol. Chem.*, **237**, 254 (1935); W. W Pigman, *J Research Natl. Bur Standards*, **30**, 159 (1943).

[61] W. W. Pigman, *J. Research Natl. Bur. Standards*, **30**, 257 (1943); *Advances in Enzymology*, **4**, 41 (1944).

TABLE II

Provisional Classification of the Carbohydrases

Enzyme class	Other or older names for members of class	Substrates[a]
Glycosidases hydrolyzing simple glycosides and oligosaccharides[b]		
β-Glucosidases	Emulsin, cellobiases, gentiobiases, prunase, probably β-glucuronisidases	β-D-Glucosides, β-D-xylosides, cellobiose, gentiobiose, β-D-glucuronides (?) heptosides with β-D-glucose configuration (?)
α-Glucosidases	Maltases, probably trehalases	α-D-Glucosides, α-D-xylosides (?), maltose, trehalose, and probably sucrose, heptosides with α-D-glucose configuration (?)
β-Galactosidases	Lactases	β-D-Galactosides, α-L-arabinosides, β-D-fucosides (?), lactose, heptosides with β-D-galactose configuration, β-D-galacturonides (?)
α-Galactosidases	Melibiases	α-D-Galactosides, β-L-arabinosides, α-D-fucosides (?), melibiose, heptosides with α-D-galactose configuration
β-Fructofuranosidases or invertases	Sucrase, saccharase, β-h-fructosidase	β-Fructofuranosides, sucrose, possibly inulin.
α-Mannosidases		α-D-Mannosides, α-D-lyxosides, heptosides with α-D-mannose configuration
β-Thioglucosidases	Myrosin	β-Thioglucosides, thioxylosides (?)
Nucleosidases		N-Glycosides (?) (particularly D-ribose derivatives), nucleosides
Polysaccharidases[b]		
Amylases Saccharifying amylases (with but slight liquefying power)	Diastases β-Amylases of wheat, barley, soybeans, etc	Starches, glycogen Unbranched chains of maltose residues

[a] As far as known, the substances followed by question marks have not been tested, at least not under sufficiently drastic conditions to indicate "unhydrolyzability." The others have been tested for at least one member of the class, usually the corresponding enzyme of almond emulsin.

The list of substrates is not complete but it is intended to represent the more important types.

[b] There is probably overlapping in the action of the two main classes of carbohydrases but, in general, the action of the one group of enzymes on the substrates of the second class appears to be small and usually may be neglected.

<div align="center">TABLE II—Continued</div>

Enzyme class	Other or older names for members of class	Substrates[a]
Polysaccharidases[b]		
Liquefying amylases	Dextrogenic amylases	
1. With considerable saccharifying ability	Malt and certain other cereal α-amylases; *Aspergillus* amylases	Starch substances and probably glycogen
2. With slight saccharifying ability	Pancreatic amylase, *Bacillus macerans* amylase, *Bacillus mesentericus* amylase, possibly salivary amylase	Starch substances and probably glycogen.
Phosphoamylases		
1. Phosphorylases of Hanes and Cori		Starches and glycogen by processes involving phosphorylation
2. Disaggregating amylase of Waldschmidt-Leitz and Mayer		Starches are broken by process involving phosphorylation into large molecules. (Existence is questionable.)
Inulases (possibly identical with invertases)		Inulin and other poly-β-fructofuranosides
Cellulases		Cellulose and probably other poly-β-glucosides
Hexosanases, pentosanases, etc	Cytases, hemicellulases	Hexosans, pentosans, etc.
Chitinases		Chitin and related substances
Pectic enzymes		Pectic substances.
Protopectinases		Hydrolyze native pectins *in situ* to soluble pectins
Pectinases		Pectins and polygalacturonides
Pectase		An esterase hydrolyzing ester linkages of pectins and of esterified polygalacturonides

If $[S]$ is much greater than e (or $[ES]$) as is usually the case:

$$[S] = [A] \tag{5}$$

The equilibrium constant for Reaction (1) is given by:

$$[E][S]/[ES] = K_m \tag{6}$$

or,

$$[ES] = [E][S]/K_m \tag{6}'$$

By substitution of Equation (4) in (6'):

$$[ES] = (e\text{-}[ES])\,[S]/K_m$$

Solving for the concentration of the enzyme-substrate compound, $[ES]$:

$$[ES] = \frac{e[S]}{K_m + [S]} \tag{7}$$

If Equation (2) represents the rate-determining reaction, the velocity of the reaction is given by:

$$v = k\,[ES] \tag{8}$$

In dilute solution, the water concentration remains constant and can be neglected. Substitution of Equation (7) in (8) gives:

$$v = \frac{k \cdot e \cdot [S]}{K_m + [S]} \tag{9}$$

If V is the velocity when $[S]$ is much larger than K_m (i.e., at high substrate concentrations), then:

$$V = ke, \text{ and } k = V/e \tag{10}$$

Equation (9), then becomes:

$$v = \frac{V[S]}{K_m + [S]} \tag{11}$$

or,

$$K_m = [S]\left(\frac{V}{v} - 1\right) \tag{12}$$

K_m, as may be seen from Equations (1) and (6), is the dissociation constant of the enzyme-substrate compound (ES) and has a characteristic value for each enzyme. It is known as the Michaelis constant, and the reciprocal $1/K_m = K_M$, is termed the association constant. Equation (12) has been used extensively for the calculation of enzymic dissociation constants, but the method has been much improved by Lineweaver and Burk[65] who employ the reciprocal of Equation (11) for the calculation:

$$1/v = (K_m/V[S]) + 1/V \tag{13}$$

This equation is of the form $y = ax + b$, where $y = 1/v$, $a = K_m/V$, $x = 1/[S]$ and $b = 1/V$. The plot of $1/v$ versus $1/[S]$ should yield a straight line with the y intercept as $1/V$ and the slope as K_m/V. The enzyme dissociation constant is determined by measurement of the initial velocity

[65] H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).

$(k [S])$ of decomposition of the substrate at different initial substrate concentrations. From the plot of $1/v$ against $1/[S]$, the dissociation constant K_m is calculated.

If the hydrolysis follows the first order equation, at least as a first approximation, the velocity is given by $v = k'[S]$, where k' is the observed "first-order" constant for each value of A. Substituting this relation in Equation (13), one finds:

$$(1/k')V = K_m + [S]$$ (14)

If the reciprocal of the observed first-order reaction constant is plotted against the initial substrate concentration $[A]$, for a number of experi-
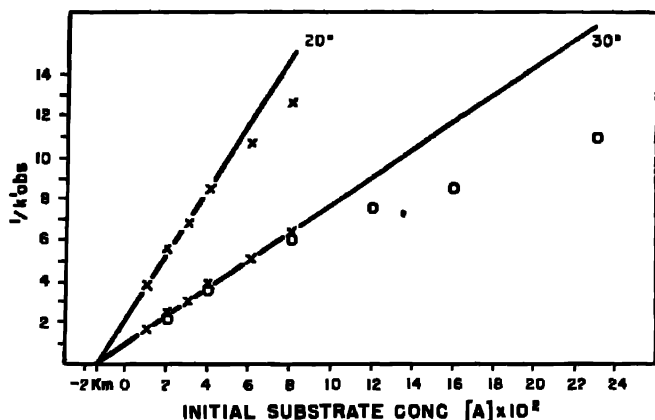
Fig. 1 Plot of concentration $(A \times 10^4)$ versus the reciprocal of the observed first order reaction constant $(1/k)$ for the hydrolysis of isobutyl β glucoside by sweet almond emulsin
(From Veibel and Lillelund.)

ments carried out at various substrate concentrations, the intercept on the A (concentration) axis, gives $-K_m$ (see Fig. 1).

This may be seen by making $1/k' = 0$, in equation (14), since then $[S] = -K_m$. It is obvious that $1/k'$ instead of $(1/k')V$ may be employed since K_m is determined under such conditions that both quantities are zero. This equation is probably the most convenient form to use for the determination of enzyme dissociation constants. This form was first suggested by Veibel,[66] but the development of the equation as given here is original.

The dissociation constants for the hydrolysis of a series of alkyl β-glucosides by the β-glucosidase of almond emulsin have been measured by Veibel and Lillelund[67] and are given in Table III.

[66] S. Veibel, Enzymologia, 3, 147 (1937).
[67] S. Veibel and H. Lillelund, Z. physiol. Chem., 253, 55 (1938).

B. Mechanism. A mechanism[68] for the action of the glycosidases is illustrated in Fig 2 This mechanism is based on the concept of the intermediate formation of a compound or complex between the enzyme nd substrate and embodies the suggestion of Euler[69] that, in the formation

TABLE III

Dissociation Constants for the Hydrolysis of β Glucosides by Sweet almond β Glucosidase

β Glucoside		K_m
CH₃		0 62
CH₃CH₂		0 25
CH₃CH₂CH₂		0 16
CH₃(CH₂)₂CH₂		0 060
CH₃(CH₂)₃CH₂		0 025
(CH₃)₂CH		0 10
CH₃(C₂H₅)CH (levo)		0 048
CH₃(C₂H₅)CH (dextro)		0 041
(CH₃)₂CHCH₂		0 017
(CH₃)₃C		1 46
C₂H₅(CH₃)₂C		0 15
CH₃(C₂H₅)₂C		0 079
(C₂H₅)₃C-		0 057
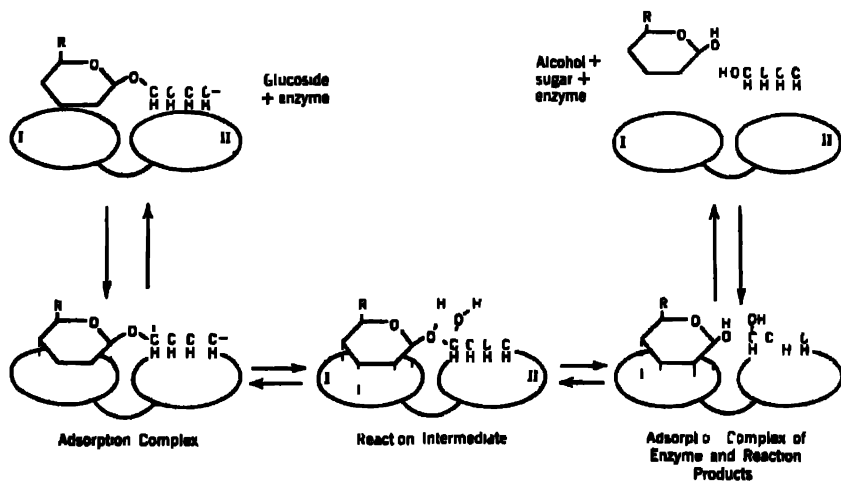
Fig 2 Postulated mechanism for the enzymic hydrolysis of an alkyl glucoside

of the enzyme-substrate complex, two areas of the enzyme molecule are involved In the figure these two areas are represented by the ovals It is

[68] W W Pigman, *J Research Natl Bur Standards*, **27**, 1 (1941), *Advances in Enzymology*, **4**, 41 (1944)

[69] H v Euler, *Z physiol Chem* , **143**, 79 (1925).

assumed that the glycoside is adsorbed[70] on these two areas, the aglycon group being taken up by area II and the sugar radical by area I. The area I exhibits extremely specific adsorption, but area II adsorbs many types of groups.

As shown in the figure, the first stage of the reaction may take place with the adsorption of the glycoside on the two areas of the enzyme surface. Next, a molecule of water (or hydronium ion) adds to the glycosidic linkage. Cleavage of the glycosidic linkage then is assumed to take place with the formation of a complex consisting of enzyme, sugar and alcohol. Dissociation of the sugar and alcohol from the surface of the enzyme comprises the final stage of the reaction.

According to this mechanism, the enzymic hydrolysis is similar to the acid hydrolysis, but through the formation of the intermediate complex, a preliminary activation of the substrate molecule takes place. The activation energy required in the second phase of the reaction, which corresponds to the reaction which takes place during acid hydrolysis, therefore is lowered. Thus, the activation energy for the acid-catalyzed hydrolysis of methyl β-glucoside is 32,610 cal. as compared with only 12,200 cal. for the enzyme catalyzed reaction.[71] The total activation energy may be considered to be derived from two sources: (1) from the formation of the enzyme-substrate complex, and (2) from the addition of the solvent or hydronium ion. During the period of combination of the enzyme and glycoside, which probably is very short, the translational and the vibrational energies of the substrate molecule are restricted and may be one source of energy during the preliminary activation. The substrate molecule primarily is the source of this energy. However, activation may also result from molecular distortion or "straining" of the substrate molecule. Thus, it might be considered that in the enzyme-substrate complex, the two components of the glucoside would be kept further apart than corresponds to the normal equilibrium distance in the free glycoside. Also, if the two active areas on the enzyme move relative to one another, the substrate molecule would be "strained." For such activation, the source of the necessary energy would be the thermal energy of the enzyme.

The mechanism described in Fig. 2 will account in a quantitative or qualitative fashion for most of the characteristics of enzyme-catalyzed

[70] The term adsorption is used in a very general sense, and the combination may take place through hydrogen and electrostatic bonds, van der Waals forces and possibly even weak covalent bonds. As shown by Hitchcock, the same kinetic equations result from consideration of the process as the formation of a chemical compound or as a simple adsorption (D. I. Hitchcock, *J. Am. Chem. Soc.*, **48**, 2870 (1926).

[71] S. Veibel and E. Frederiksen, *Kgl. Danske Videnskab. Selskab, Math. fys. Medd.*, **19**, No. 1 (1941).

reactions and will be used in the present chapter for this purpose. As previously described, the Michaelis equation and its modifications usually account for the influence of glycoside concentration on the rate of reaction. The action of various substances such as sugars and alcohols in inhibiting the reaction also agrees with this mechanism, for they may be considered to compete with the substrate for the active areas of the enzyme. The effect of inhibitors may be written as:

Glucoside + inhibitor + enzyme ⇌ glucoside-enzyme + inhibitor-enzyme

Frequently, the inhibiting effect may be quantitatively accounted for by the calculation of the dissociation constant of the enzyme-inhibitor compound. The constant K_I is obtained from studies of the influence of the concentration of the inhibitor on the reaction constant at various substrate concentrations. It is calculated by use of the equation:

$$K_{m_I} = \frac{K_m \cdot [I]}{(K_m + [S])((k/k_I) - 1))}$$

where I is the inhibitor concentration, and k and k_I are the observed reaction constants in the absence and in the presence of the inhibitor.[72] The other terms have their usual meanings. Since the products of hydrolysis of glycosides often are inhibitors, the reaction constants calculated from the first-order equation may decrease somewhat during the reaction.

In the development of the kinetic equations it was assumed that the concentration of the solvent (water) remains constant throughout the reaction. At high substrate concentrations, this is not true, however, and there is a deviation from the theoretical equations. In the case of the inversion of sucrose by yeast invertase, the effect of the sucrose and water concentration was investigated by Nelson and Schubert[73] who found that the velocity of hydrolysis increases with substrate concentration to about 5 per cent sucrose but thereafter decreases steadily. As shown by these investigators, however, the decrease may be accounted for by the decrease in the water concentration as the sugar concentration increases.

C. Influence of Hydrogen Ion Concentration. The enzymic hydrolysis of carbohydrates and derivatives is influenced markedly by the hydrogen ion concentration. There is an optimal region of pH, and at higher and lower values the activity decreases. Fig. 3 gives the pH activity curves for the hydrolysis of sucrose, raffinose and inulin by purified yeast invertase.[74]

[72] See. S Veibel, *Enzymologia*, *3*, 117 (1937).
[73] J M. Nelson and M. P Schubert. *J. Am. Chem Soc.*, *50*, 2188 (1928)
[74] M. Adams, N. K Richtmyer and C S. Hudson, *J Am Chem Soc*, *65*, 1360 (1943).

An explanation[75] for the influence of the hydrogen ion concentration is that these enzymes are amphoteric and that only the undissociated molecule is catalytically active; on this basis, equations have been developed which express quantitatively the effect of the pH. This explanation also applies to the mechanism of Fig. 3. As another possible explanation, the effect may be considered to be due to the opposing influence of two factors. Thus, on the alkaline side of the catenary, the rate increases with increase of acidity. This behavior would be expected if hydrogen ion is a catalyst for the reaction. However, an opposing factor would be the competition of hydrogen ions for the adsorbing groups in the active areas of the enzyme. At acidities on the alkaline side of the catenary, it would be expected that the dissociation constants would be essentially independent of the hydrogen
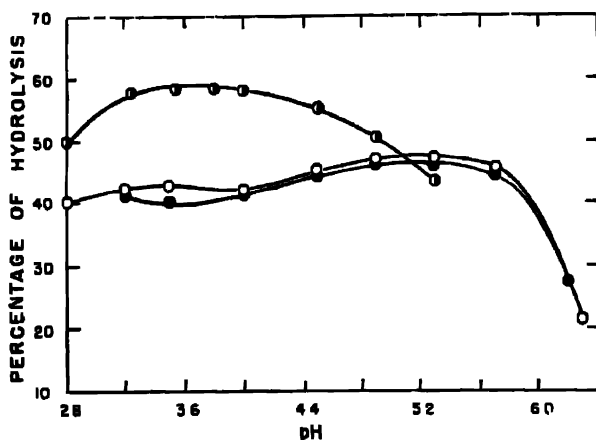
Fig. 3. pH-Activity curves for the hydrolysis of sucrose, (circles), raffinose (filled circles) and inulin (half-filled circles).

ion concentration; on the acid side there would be a marked increase in the dissociation constants (less association). Unfortunately, there are not sufficient good data to test this latter interpretation.

D. Measurement of Activity and Influence of Enzyme Concentration. As shown by Equation (9) (given earlier in this Chapter), the initial velocity at any fixed substrate concentration is directly proportional to the enzyme concentration. In general, the velocity constant calculated according to the first-order equation exhibits a similar relation as would be expected because $v = k_{obs}.[S]$. Table IV shows the results obtained for different concentrations (g) of *Aspergillus niger* emulsin with sucrose as the substrate.[76] Since the reaction constant often varies somewhat with the degree of hydrolysis, the extrapolated initial value should be used or several

[75] L. Michaelis and M. Rothstein, *Biochem. Z.*, *110*, 217 (1920).

values over the range 30 to 50% hydrolysis should be averaged. The k/g ratio usually may be employed for the expression of enzyme activity under carefully specified experimental conditions. Weidenhagen[76,77] has suggested a set of standard conditions which it would seem well to adopt until more favorable conditions are known. In general, he proposes the use of 0.1388 M substrate solutions at the optimal pH and at 30°C. The enzyme concentration is taken as the grams of emulsin or pure enzyme present in 50 ml. of reaction mixture. Reaction constants calculated from the first-order equation over the interval 30 to 50 per cent hydrolysis are used to calculate the activity from the relation:

$$E V = \text{enzyme value} = \frac{k}{g \cdot \log 2}$$

The enzyme value thus obtained is the reciprocal of the time for 50 per cent hydrolysis under the given conditions and with one gram of emulsin in 50 ml. For several of the important glycosidases, the following substances

TABLE IV

Influence of Enzyme Concentration on the Reaction Constant

Emulsin Concentration (g 50 ml)	$k \times 10^4$ (First Order Equation)	$k_1 \times 10^4$
0 138	131	300
0 0438	13 1	300
0 00876	2 66	300
0 00438	1 09	250

have been selected as the standard substrates: maltose (α-glucosidase), salicin (β-glucosidase), melibiose (α-galactosidase), lactose (β-galactosidase) and sucrose (invertase).

Because many substrates are too insoluble for the "standard conditions" to be employed and because many glycosides are available in only small quantities, it is common in specificity studies to use smaller concentrations. Particularly, in the investigation of the specificity of β-glucosidase, 0.052 M glucoside concentrations have been employed. The other experimental conditions are the same, however. The same formula is used for the calculation of activity, which in this case is called the enzyme efficiency or "Wertigkeit."

E. Temperature Influences. The rate of an enzyme-catalyzed reaction increases as the temperature is raised above room temperature, but, in

[76] W. W. Pigman, *J. Research Natl Bur. Standards*, *30*, 159 (1943)

[77] R. Weidenhagen, in "Handbuch der Enzymologie," p. 538; Editors F F Nord and R. Weidenhagen, Akademische Verlagsgesellschaft, Leipzig (1940).

contrast to ordinary chemical reactions, the rate reaches a maximum and finally falls off as the temperature continues to increase. This decrease in rate usually arises from the destruction of the enzyme at the higher temperatures. Although "optimal temperatures" are given in the literature for some enzymes, these temperatures are not true constants. They will vary according to the conditions employed, e.g., according to the relative amounts of enzyme and substrate.

In the region of the increase of reaction constants with increase of temperature, the rates of increase for the enzyme-catalyzed reactions are less than those for the corresponding acid-catalyzed reactions. This difference in rates is reflected by the smaller values for the activation energies for the reactions brought about by enzymes as compared with those catalyzed by acids. In Table V, the activation energies are given[78] for the hydrolysis of a number of β-glucosides by the β-glucosidase of almond emulsin.

TABLE V

Comparison of Activation Energies for the Hydrolysis of β-Glucosides by β Glucosidase and by Acid

(Data of Veibel and Frederiksen)

β Glucoside	Activation Energy (calories/mole)	
	Enzymic Hydrolysis	Acidic Hydrolysis
CH₃—O—Gl	12,200	32,600
CH₃CH CH₂—O— Gl	13,500	32,400
(CH₃)₂CH —O—Gl	13,100	32,100
(C₂H₅)₂CH O - Gl	10,600	31,500
(CH₃)₃C O Gl	20,000	30,800
(CH₃) C(CH₃) —O— Gl	20,000	29,900

The activation energies are calculated from the observed reaction rates at several temperatures by use of the modified form of the integrated Arrhenius equation:

$$Q = RT_1T_2(\ln k_1 - \ln k_2)/(T_1 - T_2)$$

(Q = activation energy,

k_1 and k_2 = reaction constants at the temperatures T_1 and T_2,

R = gas constant)

The activation energy may be pictured as the amount of energy required to bring one mole of molecules into a condition ("activated state") such that decomposition into the reaction products can take place. A possible mechanism for this action is discussed earlier in this chapter.

[78] S. Veibel and E Frederiksen, *Kgl. Danske Videnskab Selskab , Math. fys. Medd., 19*, no. 1 (1941).

3. Chemical Composition of Glycosidases

Unfortunately, crystalline glycosidases are still unknown, but a highly purified β-glucosidase and an invertase (β-fructofuranosidase) have been described.

A. β-Glucosidase. From almond emulsin, enzymes with a β-glucosidase value as high as 16 have been prepared. (The ordinary almond emulsins have a value of 1 or less.) The properties of such preparations have been studied by Helferich and associates.[79] The elementary composition agrees with that of protein substances, but the hydrogen content is somewhat greater than usual. The protein nature of the material also agrees with the action of the anions of neutral salts on the enzymic activity since the order of activation by these anions is similar to the well known lyotropic series. The inactivation of the enzyme by formaldehyde may also be due to its protein character.

Against neutral reducing agents, the enzyme is quite resistant, but oxidizing agents destroy it rapidly. Hydrocyanic acid, hydrogen sulfide and glutathione have no destructive action, but ozone and osmium tetroxide as well as ultraviolet light bring about rapid inactivation. A quantitative investigation of the ozone inactivation gives an equivalent weight of 800 to 1100 for the enzyme. Since the absorption spectrum of the enzyme and the rate of inactivation by ozone are similar to those for tryptophan, it is possible that this amino acid constitutes an important part of the enzyme structure. The destructive action of osmium tetroxide seems to be of a different character from the action of ozone since it does not parallel the tryptophan destruction and since a considerable part of the activity of the oxidized enzyme may be restored by reduction with hydrogen sulfide or cysteine.

Even highly purified β-glucosidase preparations contain 3 to 6% of carbohydrate material as estimated by the orcinol reaction, but there seems to be no direct correlation between the activity and the carbohydrate content.[80] The carbohydrate constituent is of particular interest since Helferich[81] has suggested that it may constitute the holding group ("Haftstelle") of the enzyme and be responsible for the adsorption of the glycoside. In terms of the mechanism proposed earlier in this chapter, this suggestion means that the very specific area (I) may consist of a carbohydrate group built into the protein structure. Adsorption of the sugar radical of a glycoside then would be analogous to the deposition of molecules on seed nuclei in a supersaturated solution. Although such a role for the carbohydrate

[79] B. Helferich, *Ergeb. Enzymforsch* , **7**, 98 (1938)

[80] B. Helferich and W. W. Pigman, *Z. physiol. Chem* , **259**, 253 (1939).

[81] B. Helferich, W. Richter, and S. Grunler, *Ber. Verhandl. sachs. Akad. Wiss. Leipzig, Math. phys. Klasse*, **89**, 385 (1937).

would explain the marked influence of changes in the sugar radical of the glycoside on the rate of hydrolysis, the concept admittedly is without much experimental foundation.

In view of the important role of dialyzable coenzymes in certain enzyme systems, it is important to note that there is no evidence for a coenzyme playing a part in the hydrolysis of β-glycosides. When solutions of purified β-glucosidase buffered at various pH's between 2.75 and 10.35 are dialyzed, the activity of the solution remains the same as that of a similar un-dialyzed solution. Although a decrease of activity takes place at the greater acidities and alkalinities, this decrease is due to an irreversible destruction of the enzyme. In confirmation of this conclusion, the addition of material passing through the membrane or of boiled enzyme does not restore the activity.[82] The β-glucosidase activity is destroyed by treatment with diazomethane, with trypsin and by acetylation.[83] The enzyme is most stable at pH 6 to 7.5; at pH 9.5 and 3.5, solutions of the enzyme are appreciably inactivated after several days at 30°C.[82,84]

B. Yeast Invertase. Several reports of the preparation of highly active yeast invertase preparations have been made. The activity of these preparations is such that the time values are less than 0.2 minute. (A time value of 0.2 minute corresponds to an enzyme value of about 700; commercial invertases usually have enzyme values of 10 or less.) Those of Adams and Hudson[45] are reported to give typical protein reactions as well as a positive Molisch test for carbohydrates. The most active preparations contain only 6.9% of reducing material after an acid hydrolysis, but others contain as much as 30 to 50% carbohydrate. Invertase preparations with large carbohydrate contents fail to give the usual protein precipitation tests and are not coagulated by heating. Although such preparations are of appreciably less activity than those with smaller carbohydrate contents, the activity of all is closely the same when based on the nitrogen content.

4. Enzymes of Almond Emulsin

A. Preparation and Purification. Almond emulsin is prepared from de fatted crushed almonds by extraction with water and precipitation of the extracts with alcohol. The dried powder is known as almond emulsin. A preparation of considerably more activity is obtained by treatment of the almond meal with zinc sulfate solution and precipitation of the enzymes from solution by the addition of tannin. The tannin is separated from the enzyme by extraction of the precipitate with acetone, and the solid residue

[82] B. Helferich, R. Hiltmann and W. W. Pigman, Z. physiol. Chem., 259, 150 (1939).

[83] B. Helferich, P. E. Speidel and W. Toeldte, Z. physiol. Chem., 128, 99 (1923).

[84] B. Helferich and A. Schneidmüller, Z. physiol. Chem., 198, 100 (1931).

[45] M. Adams and C. S. Hudson, J. Am. Chem. Soc., 65, 1359 (1943).

is the "Rohferment" of Helferich[86] which has been used for many of the studies of the specificities of the enzyme components of almond emulsin. The "Rohferment" usually has a β-glucosidase value of about 1.

Purification of the "Rohferment" gives preparations 10 to 16 times more active (β-glucosidase value 10 to 16) and some of the enzyme constituents of the cruder preparations are lost in the purification process. It should be noted that almond emulsin is not a definite substance but is a mixture of enzymes which are present in variable proportions depending on the method and extent of purification. Various methods for the purification may be used singly or in combination. The preparations of highest activity[87] have been obtained from the "Rohferment" by adsorption of the enzymes on silver hydroxide and, after desorption by action of ammonium sulfide, removal of certain of the impurities by their preferential adsorption on activated carbon.

B. Optimal pH. According to the measurements of K. Hill,[88] the maximal activity for almond β-glucosidase is shown at pH 5.0 and for α-mannosidase at 3.3 to 5. The position of the pH optimum for the β-galactosidase exhibits an influence of purification; thus, the "Rohferment" shows its maximal activity over a broader interval (pH 3.3 to 7) than that for the purified product (pH 5 to 6) (see Fig. 4).

The influence of buffer type and concentration on the optimal pH for β-glucosidase activity has been investigated by Veibel and Lillelund[89] using the o-cresyl and n-butyl β-glucosides. The optimal pH for the hydrolysis of these glucosides varies between 4.0 and 5.0. The hydrolysis takes place more rapidly in citrate than in acetate buffered solution. The two glucosides exhibit an appreciable difference in the optimal pH for the solutions in acetate buffers. The hydrolysis rates in acetate-buffered solutions are affected by the concentration of the buffer to an extent of about 10% in the range 0.04 to 0.12 M although there is little effect of buffer concentration to be observed for the citrate-buffered solutions.

C. Enzymes Present. In addition to β-glucosidase, the enzyme which hydrolyzes β-glucosides, other enzymes are present in the "Rohferment" and in the purified preparations. The methods used for establishing the presence of different enzymes in the emulsins are based on the production of changes in the relative rates of hydrolysis of several glycosides as a result

[86] B. Helferich, S. Winkler, R. Gootz, O. Peters and E. Gunther, *Z. physiol. Chem.*, *208*, 91 (1932).

[87] B. Helferich and S. Winkler, *Z. physiol. Chem.*, *209*, 269 (1932); B. Helferich and W. W. Pigman, *Z. physiol. Chem.*, *259*, 253 (1939); B. Helferich and M. Hase, *ibid.*, *274*, 261 (1942).

[88] K. Hill, *Ber. Verhandl. sächs. Akad. Wiss. Leipzig, Math. phys. Klasse* *86*, 115 (1934).

[89] S. Veibel and H. Lillelund, *Enzymologia*, *9*, 161 (1940).

of some treatment given to the enzyme preparation. Thus, in the purification of crude almond emulsin, the activity towards β-glucosides and β-galactosides increases but that towards α-galactosides decreases. This change of activity is taken as evidence for the presence of a special enzyme (α-galactosidase) different from the β-glucosidase. Also when solutions of almond emulsin are heated or exposed to ultraviolet light, their ability to hydrolyze α-mannosides may be unaffected although the ability to hydrolyze β-glucosides may be greatly diminished. Hence, the existence of a special α-mannosidase is postulated. By similar methods, the presence of
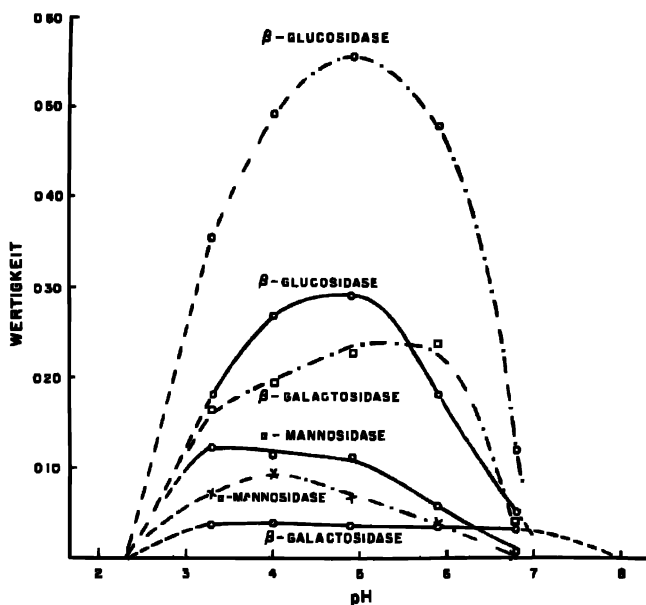
Fig. 4. The relation of pH to the activity of the principal enzymes of almond emulsin. The full lines give data for the "Rohferment" and the broken lines data for the highly purified emulsin. The upper curve for purified β glucosidase has been constructed at one quarter of its real value
(Data of K. Hill)

two other glycosidases is indicated. These enzymes are β-glucuronidase and β-(N-acetyl)-glucoaminidase which hydrolyze the β-glycosidic derivatives of glucuronic acid and (N-acetyl)-glucosamine. The enzyme preparations also catalyze the cleavage of β-galactosides. There is as yet no definite evidence that β-galactosidase differs from β-glucosidase. In the subsequent discussion, however, they will be considered to be different.

The four principal glycosidases in almond emulsin (β-glucosidase, β-galactosidase, α-galactosidase and α-mannosidase) are probably responsible for the hydrolysis of the pentosides and some other glycosides. Of the pentosides, the L-arabinosides, the β-D-xylosides, and α-D-lyxosides are

hydrolyzed by almond emulsin. The D-arabinosides and the β-L-xylosides are not affected, and the D- and L-ribosides and L-lyxosides have not been tested. It is of considerable interest that the hydrolyzable pentosides are configurationally related to hydrolyzable hexosides (i.e., they belong to the same homomorphous series) and may be considered to be derived from them by substitution of the primary alcoholic groups by hydrogen atoms (see Chapter II).

α-D-Mannose type: α-D-mannose, α-D-lyxose

β-D-Galactose type: β-D-galactose, α-L-arabinose

α-D-Galactose type: α-D-galactose, β-L-arabinose

β-D-Glucose type: β-D-glucose, β-D-xylose

Since L-arabinose and D-xylose are found in natural products, it would be possible that special L-arabinosidases and D-xylosidases exist; the existence of a special D-lyxosidase seems improbable since the sugar is not a natural product. In the case of the D-lyxosides, the hydrolysis most probably is due to the α-mannosidase component of almond emulsin.[90] The stability of the enzyme to heat supports this conclusion. The simplest explanation for the hydrolysis of the other pentosides is that they also are cleaved by the corresponding hexosidases. This concept receives support from studies of the effect of purification on the relative rates of hydrolysis of the hexosides and pentosides.[86] Thus, purification of the cruder preparations of almond emulsin increases their ability to hydrolyze α-L-arabinosides and β-D-xylosides in the same relative proportions as for the configurationally related β-galactosides and β-glucosides. The α-galactosidase activity decreases, however, in the same proportion as the ability to catalyze the cleavage of the β-L-arabinosides. Although this evidence does not eliminate the possibility of special pentosidases, particularly in enzyme preparations from other sources, it makes their postulation in almond emulsin unnecessary.

It has never been conclusively demonstrated that only a single component in almond emulsin is responsible for the cleavage of all β-glucosides, i.e., that several β-glucosidases are not present, but most of the available evidence supports this concept. Apricot emulsin as well as three almond emulsins of different purity were investigated[91] for their action on β-glucosides which had the following substances as the aglycons: methanol, glucose, phenol and saligenin. As shown in Table VI, the relative rates of hydrolysis of the series of glucosides by the four enzyme preparations is the same within the experimental error. The earlier work of Willstätter, Kuhn and Sobotka[92] led to the same conclusion. However, later work,[93]

[90] W. W. Pigman, J. Am. Chem. Soc., 63, 1371 (1910).

[91] B. Helferich, R. Gootz and G. Sparmberg, Z. physiol. Chem., 205, 201 (1932).

[92] R. Willstätter, R. Kuhn and H. Sobotka, Z. physiol. Chem., 129, 33 (1923).

[93] L. Zechmeister, G. Tóth, P. Furth and J. Bársony, Enzymologia, 9, 155 (1941).

which should be checked, indicates that several β-glucosidases may be present.

D. Specificity of the β-Glucosidase. *Influences of substitution and of configurational changes in the sugar radical of glucosides.* A number of β-glucosides have been prepared in which the hydroxyl groups on carbons 2,3 and 4 have been substituted by methoxyl and tosyloxy groups. For all such compounds, the derivatives have been found to be "unhydrolyzable."[91] Thus, the 3-methyl and the 2,4,6-trimethyl derivatives of phenyl β-glucoside as well as the 2-tosyl, 3-tosyl and 4-tosyl derivatives of vanillin β-glucoside are not appreciably hydrolyzed by almond emulsin even after 100 hours or more.[95,96]

When substitutions are made at carbon 6 of β-glucosides, the effects are quite different than those arising from substitutions at the ring carbons (2, 3, 4, 5). In the accompanying formula, this type of substitution is

TABLE VI

Effect of Purification on the Rates of Hydrolysis of a Series of β-Glucosides

β Glucoside	Apricot Emulsin	Enzyme Value (or Relative Enzyme Value)		
		Almond Emulsin		
		I	II	III
Methyl β glucoside	0 013 (1)	0.012 (1)	0 021 (1)	0 13 (1)
Cellobiose	0 031 (2.6)	0.010 (3 3)	0 052 (2 5)	0 27 (2 1)
Phenyl β glucoside	0.076 (5 8)	0 070 (5 8)	0 13 (6 2)	0 67 (5 2)
Salicin	0 44 (32)	0 12 (28)	0 67 (32)	3 4 (26)

represented by variations in the nature of the atoms or groups represented by X.
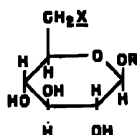
The ease of hydrolysis of a series of such 6-substituted β-glucosides[97] is compared in Table VII.

[91] In most instances, under stringent experimental conditions utilizing high enzyme concentrations and long time periods, the minimum enzyme efficiency ("Wertigkeit") which is significant is about 10^{-5}. This is about 1/33,000 the rate of hydrolysis of a fairly easily hydrolyzable glucoside (phenyl β-glucoside with E. E. = 0.33). A compound with enzyme efficiency of 10^{-5} or less is usually considered to be "unhydrolyzable."

[95] B. Helferich and S. Grünler, *J. prakt. Chem.*, [2] *148*, 107 (1937)

[96] W. W. Pigman and N. K. Richtmyer, *J. Am. Chem. Soc.*, *64*, 374 (1942).

[97] B. Helferich, S. Grünler and A. Gnüchtel, *Z. physiol. Chem.*, *248*, 85 (1937)

In general there seems to be a correlation between the size of the group X and its influence on the rate of hydrolysis as may be seen by a comparison of the rates of hydrolysis and the volumes of the groups as given by Biltz. It appears that the effect of groups substituted for the hydroxyl of carbon 6 is a steric effect, and that such substitutions produce only quantitative changes in the rate of hydrolysis.[96,97] However, when the substituent groups are quite large, e.g., benzoyl and tosyl groups, the rate of hydrolysis becomes so slow as to be immeasurable. It is of interest that apparently the most easily hydrolyzed glycoside types are those with $X = H$, i.e., the 6-desoxyglucosides.

A similar type of substitution is the replacement of the primary alcohol group ($-CH_2OH$) of hexosides by H or $CHOH-CH_2OH$ to form the pentosides or heptosides of similar ring configuration. The evidence available

TABLE VII

Comparative Rates of Hydrolysis of 6-Substituted β Glucosides

Substituent Group (Group X)	Phenyl 6-X-β glucoside (E E)	Vanillin 6 X-β glucoside[a]	Volume of Group X^b (Biltz)
H	0 56		5 8
OH	0.3	39	9.4
F	0.03	(6)	15 9
Cl	-	2.5	16.5
Br	0 003	1.5	19.5
OCH₃	0 0023		21 1
I		0 17	24 3

[a] Calculated in the same manner as for the enzyme efficiencies but employing 0 00104 M substrate solutions.

[b] Molecular volume in milliliters.

for believing that the α- and β-L-arabinosides, the β-D-xylosides and α-D lyxoside are acted on by β- and α-galactosidase, β-glucosidase and α-mannosidase, respectively, has been cited previously. The preparation of the phenyl D-manno-β-D-*galacto*-heptoside (phenyl β-D-α-mannoheptoside) has made it possible to test the influence of the substitution of a $-CH_2OH$ group for the hydrogen attached to carbon 6 on the ease of hydrolysis of phenyl β-galactoside. As would be expected from the presence of the β-galactopyranoside ring, this heptoside is hydrolyzed by almond emulsin at a slow but significant rate[98] ($E.E. = 0.00022$ as compared with 0 032 for phenyl β-galactoside).

Changes of Ring Structure and Configuration. When the structure of a hydrolyzable pyranoside is changed to that of a furanoside, the latter is unaffected by the enzyme. Thus, the α- and β-D-glucofuranosides are un-

[96] W. W. Pigman, *J. Research Natl. Bur. Standards*, **26**, 197 (1941).

affected[99] by almond emulsin as well as by yeast α-glucosidase (yeast maltase). Similarly, yeast invertase catalyzes the cleavage of sucrose and other β-fructofuranosides, but not that of the known fructopyranosides.[100]

The influence of the configuration of the carbons forming the pyranose rings of hydrolyzable glycosides has also been investigated. In no known instance is the enantiomorphous modification of a hydrolyzable glycoside acted upon by the same enzyme. The best examples, at present, are the pairs of β-D- and β-L-arabinosides and the α-D- and α-L-arabinosides. Although the L-isomers are easily cleaved, the D-isomers remain unaffected even after long periods of time.[98,101] A similar difference exists for the hydrolyzable D-xylosides and the unhydrolyzable L-xylosides.[102] The earlier work of E. Fischer showed that the L-glucosides are unaffected by enzymes, but these results require confirmation.

It is of interest to consider the effect of variations in the configuration of a single asymmetric center. Data are available for making this comparison as a result of studies of the action of almond emulsin on the phenyl α-D-taloside and the methyl D-gulosides, none of which was found[98,103] to be appreciably hydrolyzed. Inasmuch as the α-D-talosides differ from the hydrolyzable α-D-mannosides only in the configuration of a single carbon atom (carbon 4), it seems that the change in configuration of only a single asymmetric center is sufficient to prevent enzymic action. This is substantiated by the lack of enzymic cleavage of the gulosides which differ from the hydroyzable galactosides only in the configuration of carbon 3.

The experiments cited illustrate the extreme specificity of the almond emulsin enzymes to slight changes in the sugar portion of glycosides. At present it seems that these influences may be summarized by the statement that *hydrolyzable glycosides must belong to one of the naturally occurring types* (p. 57) *and none of the ring hydroxyls may be substituted by other groups*. Conversely, enzymes are to be expected in natural products corresponding to each of the naturally occurring types, and it is probable that no enzymes exist for the hexoside types which are not naturally occurring. However, it is not to be expected that individual enzyme preparations will contain all of the possible enzymes; instead enzymes usually will be found in biological materials in association with their substrates.

Aglycon Specificity. The marked influence of changes in the rings of hydrolyzable glycosides on enzymic action has been emphasized in the preceding section. The effect of variations in the aglycon group are quite

[99] E. Fischer, Ber., **47**, 1980 (1914); W. N. Haworth, C. R. Porter and A. C. Waine, J. Chem. Soc., 2254 (1932).

[100] R. Weidenhagen, Z. Ver. deut Zucker-Ind., **82**, 921 (1932).

[101] B. Helferich, H. Appel and R Gootz, Z. physiol. Chem , **215**, 277 (1933).

[102] B. Helferich, E. Günther and W. W. Pigman, Ber , **72**, 1953 (1939).

[103] B. Helferich, W. W. Pigman and H. S. Isbell, Z. physiol. Chem., **261**, 55 (1939)

different, however, and it is unusual to find that structural changes completely inhibit enzymic cleavage although occasionally the rate is very slow. In the mechanism described previously, the concept of a general unspecific adsorption on the area II was developed to agree with the experimental work which has been done on the influence of the structure of the aglycon group. In the following discussion of this work, the β-glucosides will be
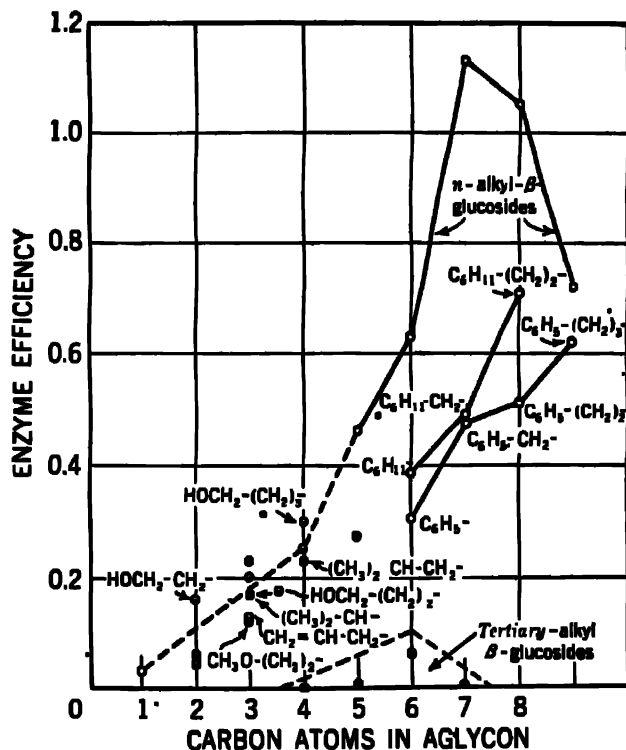
Fig. 5. The relationship between the enzyme efficiency and the number of the carbon atoms in the aglycon groups of alkyl β-glucosides. Circles represent data of Pigman and Richtmyer; filled circles are data of Veibel, and half-filled circles are data of Helferich.

divided into those yielding glucose and an alcohol on hydrolysis (alkyl β-glucosides) and those giving glucose and a phenol (aryl β-glucosides).

Alkyl β-Glucosides. The enzymic hydrolysis of numerous alkyl β-glucosides has been studied. Many of the results obtained are summarized[104] in

[104] This figure is taken from W. W. Pigman and N. K. Richtmyer, *J. Am. Chem. Soc.*, *64*, 369 (1942) and W. W. Pigman, *Advances in Enzymology*, *4*, 41 (1944). It incorporates results of Veibel and associates and some of Helferich. Some new data by Helferich and Goerdeler, *Ber. Verhandl. sächs. Akad. Wiss. Leipzig, Math. Phys. Klasse*, *92*, 75 (1940) are included. The results of Veibel at various concentrations has been interpolated at the standard concentration (0.052 *M*).

Fig. 5. It is of considerable interest that in the various homologous series the rate of hydrolysis increases with increasing chain length. The *n*-alkyl series shows a progressive increase in the rate of hydrolysis with increasing chain length of the aglycon group until the chain length reaches about 7 carbon atoms. Thereafter, however, the rate decreases. On the basis of the postulated mechanism previously given (Fig. 2), this occurrence of an optimal chain length for maximal hydrolysis is to be ascribed to a counter-balancing of the beneficial effects of increased chain length on the formation of the enzyme-substrate complex by the disadvantageous influence of slow desorption of the products of hydrolysis from the enzyme's surface. That is,
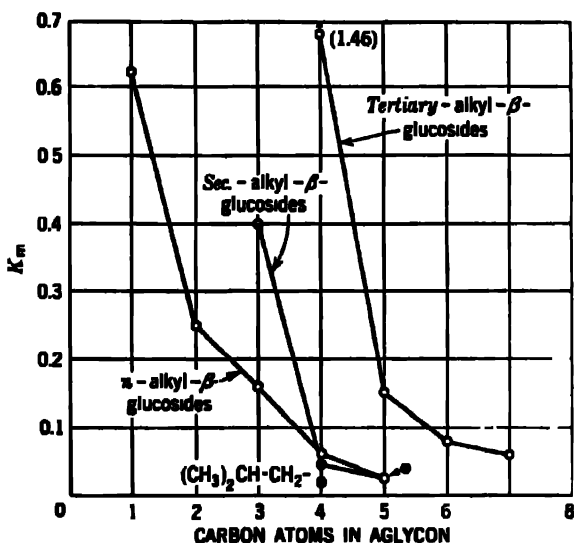
FIG. 6. Relationship between the dissociation constant of the enzyme substrate complex and the number of carbon atoms in the aglycon groups of alkyl β-glucosides.

the rate determining reaction for the *n*-nonyl glucoside may be the dissociation of the *n*-nonyl alcohol from the surface of the enzyme rather than the decomposition of the glucosidic linkage.

This explanation of the influence of increasing chain length or molecular weight of the aglycon receives support from a comparison of the dissociation constants of the enzyme-alkyl glucoside complex with the number of carbons in the aglycon group. In Fig. 6, such a comparison is made using the data reported by Veibel and Lillelund.[106] In the various series, the adsorption increases rapidly with the increasing size of the aglycon group. The increase in adsorption is indicated by a decrease in the dissociation

[106] S. Veibel and H. Lillelund, *Z. physiol. Chem.*, **253**, 55 (1938).

constants. The highest association (adsorption) is shown by the *n*-alkyl series and the association becomes less as the extent of branching increases. This influence of branching might be expected as the straight chains should have a greater opportunity to accommodate themselves to the adsorbing atoms of the active areas on the enzyme. A summary of the effect of various groups in the aglycon radicals of alkyl β-glucosides and additional discussion is given elsewhere.[62]

TABLE VIII

Rate of Enzymic Hydrolysis of Disaccharides and Related Compounds with β Glucosidic and β-Galactosidic Linkages

	β Glucosides			β-Galactosides		
Substrate	Structure	$E.E. \times 10^6$		Substrate	Structure	$E.E. \times 10^6$
Cellobiose	4 Glucose β gluco-side	159, 180		Lactose	4-Glucose β-galac-toside	11 2
Gentiobiose	6 Glucose β-gluco-side	75		Lactulose	4 Fructose β-galac toside	(14)
Celtrobiose	4 Altrose β-gluco-side	(23)		Neolactose	4 Altrose β-galacto-side	(2 8)
	4 Mannose β-gluco-side	2 3		Lactositol	4 Sorbitol β-galac-toside	0.84
Phenyl α cel lobioside	4 (Phenyl α-gluco side) β-glucoside	160		Lactobionic acid	4-(Gluconic acid) β-galactoside	0 41
Phenyl β glu coside		330		Phenyl β-lactoside	4 (Phenyl β-gluco-side) β-galacto-side	23
				Protocate-chuic alde-hyde β-lac-toside	4-(Protocatechuic aldehyde β-gluco-side) β-galacto-side	80
				Phenyl β-galacto-side	Phenyl β galacto-side	32–49

In Table VIII, values are given for the ease of hydrolysis of some disaccharides which have β-glucosidic or β-galactosidic linkages.[63] For comparison, several other glycosides are included.
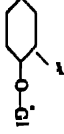
The marked influence of the effect of structural and configurational changes in the aglycon group is very evident. Thus, cellobiose and 4-mannose β-glucoside differ only in the configuration of a single carbon atom (carbon 2 of the aglycon group) yet there is more than a 60-fold difference in the ease of hydrolysis of the two compounds. Gentiobiose and cellobiose differ only in the position of attachment of the aglycon group, but the latter is hydrolyzed at more than double the rate of the gentiobiose

The same difference is to be observed for the disaccharides and derivatives of the β-galactoside series. Here, the effects of breaking the pyranose ring of the aglycon of lactose by oxidation to lactobionic acid or reduction to lactositol may be observed. As may be seen from the table, these compounds are hydrolyzed much more slowly than lactose. The formation of the glycosidic derivatives of lactose, even though such a change is remote

<div align="center">TABLE IX</div>

Influence of Substitutions in the Aromatic Nucleus on the Hydrolysis of Phenyl β Glucoside by Sweet almond β Glucosidase

| | Monosubstituted | | | Disubstituted | |
| | Enzyme Efficiency | | | | |
Group X	Ortho Subst	Meta Subst	Para Subst	Groups and position	Enzyme efficiency
None (H)	0.33	0.33	0.33	1 CHO (monosubstituted)	4.2
CHO	8.6	–	4.2	1 CHO, 2 OH	9.7
CH₃	4.3	0.55	0.12	4 CHO, 2 OCH₃	13
CH₃O	3.5		0.27	1 CHO, 2 C₆H₅CH₂O	2.3
CH₃CO-	3.3	—	1.1	4 CH₂ CH=CH , 2 OCH₃	1.1
CH₃CH₂	2.3		0.08	3 CHO, 6 OCH₃	11
CNCH₂-	2.0		1.2	3-CHO, 6 OC₂H₅	2.5
HOCH₂-	2.0			6 CH₃, 2-CH₄	0.1
CH₃OCO-	1.6	1.5	1.5	4-CH₃, 2-CH₃	1.4
HOCO	1.6	0.60	0.43	2 CH OH (monosubstituted)	2.0
AcNHCH₂	0.88	1.48	0.13	2 CH₂OH, 4 Cl	0.48
HO	0.56	0.17	0.050	2 CH₂OH, 4-Br	0.60
HOCOCH₂-	0.13	–	0.64	2 CH₂OH 4 I	0.62
NH₃-	–		0.055		
NO₂-	—	—	0.53		
NH₂CH·	0.036	0.05	0.027		

from the linkage undergoing hydrolysis, appreciably accelerates the hydrolysis (compare lactose, phenyl lactoside and protocatechuic aldehyde lactoside).

Aryl β-Glucosides. In Table IX are recorded the results of Helferich and associates from their study of the influence of substitution in the benzene ring of phenyl β-glucoside on the ease of enzymic hydrolysis.[62] It should be noted that β-glucosidases from some sources other than almond emulsin exhibit quite different specificities; hence, the conclusions drawn

apply only to sweet-almond β-glucosidase. The principal effects of substituent groups are summarized in the following generalizations for which phenyl β-glucoside is taken as the standard for the comparison.

(1) The rate of hydrolysis is increased by the introduction of "meta-directing groups" in any position, and aldehyde groups exhibit the greatest effect.

(2) In all cases known, the rate of hydrolysis is decreased by the introduction of an amino group in any position. Acetylation of the amino group reduces its inhibiting influence.

(3) With the single exception of the amino group, all groups, when substituted in the ortho position, increase the ease of enzymic hydrolysis.

(4) Although the "meta-directing groups" increase the ease of hydrolysis when in the para position (but less than when in the ortho position), other groups have an inhibiting action.

(5) The substitution of groups in the meta position results in influences intermediate between those of the same groups in the ortho and para positions, but the general effect is to increase the ease of hydrolysis.

(6) Compounds with two groups in addition to the glucosidic group in the aromatic nucleus usually exhibit an additive influence of the two groups. Thus, for a group like methyl, the ortho effect is strongly positive and the para effect is weakly negative. The 2,4-dimethylphenyl β-glucoside is hydrolyzed at a rate intermediate between those for the two corresponding monosubstituted compounds.

As is shown by the values given for the acid hydrolysis of some of these compounds (Table X), there appears to be no correlation between the ease of enzymic and of acid hydrolysis. If the influences of these groups were merely on the glucosidic linkage, such a parallelism might be expected.

Since the substituent groups undoubtedly exert an influence on the glucosidic linkage undergoing hydrolysis as well as on the formation and stability of the enzyme-substrate complex, the interpretation of the results is difficult. It would seem, however, that the main influences would be on the formation and stability of the enzyme-substrate complex. As the ortho position is closest to the linkage undergoing hydrolysis, the formation of weak bonds between the enzyme and groups in the ortho position would tend to facilitate hydrolysis. This influence would be less for groups in the meta position and still less for those in the para position. The inhibiting influence of the amino group might be ascribed to its ionic charge and the formation of a bond with the enzyme so stable that the dissociation of the products of hydrolysis from the enzyme would be inhibited.

5. Occurrence and Specificity of Other β-Glucosidases

Although almond emulsin is the classical source of glycosidases and has received the most study, these enzymes are widely distributed in seeds, in

animal tissues and organs and in microorganisms. Although enzymes similar to the almond-emulsin glycosidases occur in many natural products, there is no reason for assuming that enzymes from the different sources which act on the same substrates should be identical. Instead it might be anticipated that the living tissues would develop enzymes best suited for the particular glycoside or disaccharide actually being synthesized or hydrolyzed. As will be shown in the subsequent discussion, the effect of changes in the aglycon groups of glycosides on the rate of enzymic hydrolysis is quite different for enzymes from different sources, i.e., the aglycon specificities vary. Unfortunately, there is practically no information on the effects

TABLE X

Comparison of the Rates of Acid and Enzyme Hydrolysis of Some Aromatic β-Glucosides

Aglycon group	Enzyme Efficiency	Acid hydrolysis $k \times 10^4$
C_6H_5	0 31	23
2-CNCH$_2$C$_6$H$_4$	2 0	4 9
4-CNCH$_2$C$_6$H$_4$	1 2	16
2-CH$_3$COC$_6$H$_4$	3 3	110
4-CH$_3$COC$_6$H$_4$	1 1	8
2-CHOC$_6$H$_4$	8 6	9
4-CHOC$_6$H$_4$	1 2	8
2-OH + CHOC$_6$H$_3$	10	13
6-OCH$_3$ + CHOC$_6$H$_3$	11	13
2-OCH$_3$+ CHOC$_6$H$_3$	13	45
4-CH$_3$ C$_6$H$_4$	0 12	21
2-CH$_3$ C$_6$H$_4$	4 3	18
2-CH OH-C$_6$H$_4$	4 9	11

of changes in the sugar portion of glycosides on the enzymic hydrolysis for any enzymes except those from almond emulsin.

A comparison of the specific action of β-glucosidases from a number of sources on four different β-glucosides has been made by Miwa, Cheng, Fujisaki and Toishi[106] and their results are summarized in Table XI. The table records the ease of hydrolysis as "*f*" values and also as relative values with the ease of hydrolysis of the phenyl β-glucoside being taken as unity for each enzyme preparation. The apricot, sweet-almond and peach β-glucosidases are markedly affected by substitution in the ortho position as is demonstrated by the values for the salicyl and o-cresyl β-glucosides, para substitution is much less effective and inhibits the hydrolysis. The fungal β-glucosidases exhibit an inhibitory effect of ortho substitution

[106] T Miwa, C Cheng, M. Fujisaki and A. Toishi, *Acta Phytochim (Japan)*, 10 155 (1937).

those from the other plant sources seem to be intermediate in character between the two extremes, and to be much less affected by structural changes in the aglycon groups.

The digestive juices of the snail (*Helix pomatia*) contain a β-glucosidase in addition to an active cellulase. In Table XII, the action of the snail emulsin on several β-glucosides is compared with that of sweet-almond

TABLE XI

Comparison of the Specificity of β-Glucosidases from Different Sources

Source of Emulsin	β-Glucoside							
	Phenyl		Salicyl		o-Cresyl		p-Cresyl	
	f	Ratio	*f*	Ratio	*f*	Ratio	*f*	Ratio
Prunus armeniaca (apricot)	2 47	1 0	30.7	12.5	59 8	24 2	1 27	0.51
Amygdalus communis (sweet almond)	2.10	1.0	29.1	13.9	52.5	25 0	1.20	0.57
Prunus persica (peach)	0 315	1.0	3.16	10.0	5.81	18.5	0.160	0 53
Cycas revoluta (sago palm)	0.0226	1.0	0 176	7.65	0.391	17 4	0 0114	0 50
Papaver somniferum (opium poppy)	0 00459	1.0	0.0163	3 54	0.0268	5 82	0 00411	0.89
Glycine hispida (soy bean)	0 00073	1 0	0.00181	2 18	0.00071	0 97	0 00146	2 0
Cucurbita moschata (squash)	0.00013	1 0	0.00503	0.55	0 00421	0 16	0.00897	1 00
Aspergillus oryzae (Takadiastase)	0 110	1.0	0.0962	0.88	0 0139	0 13	0 0722	0.59
A. oryzae	1.058	1.0	0.843	0.80	0.013	0.12	0.740	0.71
A. niger	2.90	1 0	0.965	0.32	0 0772	0.025	4.34	1.46
Ergot	0.118	1.0	0.0523	0.35	0.0349	0 24	0.148	1.0

¹ "*f*" is a measure of the ease of hydrolysis similar to the enzyme value and enzyme efficiency

emulsin.[107] The snail β-glucosidase reacts similarly to that in almond emulsin, but the influence of variations in the aglycon structure is much less.

The β-glucosidase which occurs as an impurity in highly purified yeast invertase hydrolyzes gentiobiose, but it exhibits no activity for the hydrolysis of cellobiose although almond emulsin hydrolyzes the latter disaccharide the more rapidly. Phenyl β-glucoside is cleaved at about the same rate as gentiobiose[108] in contrast to the results for almond emulsin for which

¹⁰⁷ B. Helferich and J. Goerdeler, *Ber. Verhandl. sächs Akad. Wiss. Leipzig, Math. phys. Klasse, 92*, 75 (1940).

¹⁰⁸ M Adams, N. K Richtmyer and C. S. Hudson, *J. Am. Chem Soc., 65*, 1369 (1943).

the former glucoside is the more easily hydrolyzed. The glucoside phlorizin is only slowly acted upon by almond emulsin (in comparison with salicin) and by emulsins from cattle, horse and pig liver and intestinal mucosa and pig and cattle kidneys. However, it is easily cleaved by a preparation from horse kidneys.[109] Aqueous extracts of ground rabbit livers and kidneys exhibit considerable β-glucosidase activity, and the optimal pH is around 6; in contrast, extracts of spleens, lungs, testicles and muscles have only slight ability to hydrolyze β-glucosides.[110]

6. Alfalfa and Coffee Emulsins

The glycosidase system of alfalfa (lucerne) seeds (from *Medicago sativa*) has been studied by K. Hill.[111] It differs from sweet-almond emulsin in

TABLE XII

Comparison of the Specificities of β-Glucosidase of Snail and Almond Emulsin

β-Glucoside	Sweet Almond Emulsin		Snail Emulsin	
	k	Ratio	k	Ratio
Phenyl	0.33	1.0	0.028	1.0
Salicyl	1 7	5 2	0 031	1.2
Vanillin	13	39	0 151	5.4
p-Cresyl	0.12	0.36	0.026	0 9
o-Cresyl	4.3	13	0 035	1 3
Ethyl	0.015	0.11	0.0056	0.2

having only traces of a β-glucosidase although it has a very active β-galacto-sidase and an α-galactosidase. The α-mannosidase and the β-(N-acetyl)-glucosaminidase activities are similar to those of almond emulsin.

The optimal pH for the alfalfa β-galactosidase is close to 3.4 and the maximal range is rather short. For the α-galactosidase and the α-mannosidase, the optimal pH covers a rather broad region between 3.3 and 5.0. As is also true for almond emulsin, the α-mannosidase is more stable to heat and ultraviolet light than the other enzyme components.

A comparison of the aglycon specificity of the alfalfa and the sweet-almond β-galactosidases is made[111,112] in Table XIII. It will be noted that

[109] E. Hofmann, *Biochem. Z.*, **385**, 429 (1936).

[110] K. Aizawa, *J. Biochem. (Japan)*, **30**, 89 (1939).

[111] K. Hill, *Ber. Verhandl sächs Akad Wiss. Leipzig, Math. phys. Klasse*, **86**, 115 (1934).

[112] B. Helferich and H. Scheiber, *Z. physiol. Chem.*, **226**, 272 (1934); B. Helferich and R. Griebel, *Ann.*, **544**, 191 (1940); B. Helferich, H. Scheiber, R. Streeck and F. Vorsatz, *ibid*, **518**, 211 (1935).

the alfalfa β-galactosidase is affected less by structural variations in the aglycon and is not particularly influenced by ortho substitution.

Coffee emulsin resembles the alfalfa emulsin more closely than almond emulsin; although the β-glucosidase action is weak, the β-galactosidase, α-mannosidase and particularly the α-galactosidase activities are appreciable. In contrast to the ease of hydrolysis by almond emulsin of vanillin β-galactoside as compared with phenyl β-galactoside, there is little difference in the case of hydrolysis of the two galactosides by coffee emulsin. The coffee α-galactosidase exhibits its maximal activity over a wide range of pH, 3 to 6, but the β-galactosidase has its maximum activity near 3.5 to 4 and the α-mannosidase between 4.5 and 5.5.[113]

<div align="center">TABLE XIII</div>

Comparison of the Specificity of the β Galactosidases of Alfalfa and Almond Emulsins

Glucoside	Almond Emulsin		Alfalfa Emulsin	
	k_F	Relative k_L	k_F	Relative k_L
Phenyl β galactoside	0 040	1 0	0 17	1 0
o Cresyl "	0 69	17	0 13	0 76
p Cresyl "	0 02	0 5	0 14	0 82
Protocatechuic aldehyde galactoside	7 5	190	0 36	2 1
Vanillin "	1 35	34		
Phenyl β lactoside	0 023	0 6	0 029	0 17
Protocatechuic aldehyde β-lactoside	0 08	2 0	0 021	0 12
Lactose	0 009	0 2	0 004	0 024

7. Yeast Glycosidases

The best known glycosidase component of yeasts is β-fructofuranosidase (invertase, β-h-fructosidase, sucrase, saccharase) which hydrolyzes β-fructofuranosides including sucrose. Live yeasts provide an excellent source of invertase and are used in commercial operations for the inversion of sucrose. Alcoholic fermentation can be avoided by using high sugar concentrations of the order of 50 per cent. Invertase preparations are made by allowing yeast to autolyze in the presence of an antiseptic such as toluene, and for this purpose the invertase content of the yeasts can be increased greatly by development of the yeasts in aerated sucrose solutions.[114] According to the procedure of Adams and Hudson,[115] the yeast is allowed to auto-

[113] B Helferich and F Vorsatz, Z physiol Chem, 237, 254 (1935)

[114] R Willstatter, C D Lowry, Jr, and K Schneider, Z. physiol. Chem, 146, 158 (1925); R Weidenhagen, Z. angew Chem, 47, 583 (1934)

[115] M Adams and C S Hudson, J Am Chem Soc 65, 1359 (1943)

lyze under toluene at 25°C. for several days; the liquid is filtered and immediately dialyzed at 28 to 30°C. for several days (cellophane diaphragms). At the conclusion of the dialysis, the solutions are allowed to age one or two weeks and inert material is then thrown out by acidification to pH 3.7 3.9 with acetic acid. The final stage of purification consists of adsorption on bentonite at pH 3.6 4.1 and elution at pH 5.4-5.6. By this procedure, invertase preparations are obtained with invertase values of about 1000 as compared with values of about 10 for commercial preparations. (The time value for the best preparations was about 0.16.)

The preparation and the reaction kinetics of invertase have received a great deal of study[116] particularly by Willstätter, Kuhn, Euler, Josephson, Hudson, Myrbäck, Nelson, Weidenhagen and their associates. The specificity and properties of yeast invertase have been restudied by Adams, Richtmyer and Hudson,[104] and the following details are taken from their work. The optimal pH for the hydrolysis of sucrose and raffinose is probably around 5.0 to 5.5, although earlier workers found it to be somewhat lower (3.5 to 5.5). However, the rate of decrease toward the acid side is quite small and even at pH 2.8 the enzyme is quite active (see Fig. 3, earlier in this chapter).

As shown in Table XIV, the results of Adams, Richtmyer and Hudson indicate that under the "standard conditions," the first-order reaction constants decrease for raffinose, increase for sucrose and remain fairly constant for inulin.

Specificity studies of yeast invertase are complicated by the difficulty of preparing β-fructofuranosides, and the studies have been limited to the naturally occurring di- and oligosaccharides containing β-fructofuranoside residues. Sucrose, raffinose and stachyose have such residues in an unsubstituted condition (terminal positions) whereas melezitose has a substituted β-fructofuranoside residue (central position) Inulin is a polymeric β-fructofuranoside with 1,2' linkages. All of these compounds with the exception of the melezitose are hydrolyzed by yeast invertase preparations. For an invertase preparation from bakers' yeast with enzyme value of 791, the ease of hydrolysis of sucrose, raffinose, inulin and stachyose is in the proportion, 100:23:0.036:6.8. For a similar preparation from brewers' yeast, the proportion is 100:12.5:0.006:3.1.[104] Although it is probable that sucrose, raffinose and stachyose are hydrolyzed by yeast invertase, and that the different ratios observed for the two preparations are due to variation in the invertase molecule present in the brewers' and the bakers' yeasts, it is possible that the inulin hydrolysis may be due to another enzyme

[116] For discussions of the earlier work see J. M. Nelson, Chem. Revs., 12, 1 (1933)' R Weidenhagen, in "Handbuch der Enzymologie" (Nord Weidenhagen), p 512' Akademische Verlagsgesellschaft, Leipzig (1940).

component, an inulase. Support for the existence of a special inulase is given by the marked difference between the optimal pH for the inulin and for the sucrose hydrolysis. Thus, that for the sucrose inversion lies between 5.0 and 5.5 while that for the inulin hydrolysis lies between 3.2 and 4.0. For yeasts, the ratio of invertase to inulase activity is quite large (2,800 to as high as 28,300), but for fungal emulsins, particularly those from *Aspergillus niger*, the ratio may be as low as about 5.[117]

Bottom (or brewers') yeasts contain an enzyme which hydrolyzes melibiose, an α-galactoside, and which is absent from top (bakers' or ale) yeasts.[118]

TABLE XIV

Kinetics of Hydrolysis of Sucrose, Raffinose and Inulin by Purified Yeast Invertase Preparations

Time,	Sucrose		Raffinose		Inulin	
	Hydrolysis	$k \times 10^4$	Hydrolysis	$k \times 10^4$	Hydrolysis	$k \times 10^4$
min.	per cent		per cent		per cent	
20	26.2	66.0	—	-	22.2	54.5
22	—	-	28.7	66.0	—	—
30	37.6	68.3	36.1	64.9	—	—
40	48.0	71.0	44.6	64.1	39.6	51.7
50	57.2	73.7	—	--	48.4	57.5
55	—	--	55.8	64.1	—	—
60	64.6	75.2	—	-	54.8	57.5
75	74.2	78.5	-		—	.
80		—	68.2	62.2	-	-
140		—	85.1	59.0	--	—
150	95.0	86.7	—	-	88.2	61.9
300	100.0	--	96.9	50.5	97.4	52.8
1440	—	--	100.4	-	100.0	—

a Calculated in minutes and common logarithms by use of first-order equations

This enzyme, called α-galactosidase or melibiase, is carried along with invertase when the latter is purified, and purified brewers' yeast invertase provides a good source of the enzyme.[108] The optimal pH region is fairly broad, and a pH of 4.5 is probably the best for most substrates. For such a preparation, with a melibiase value of 4.8, the relative ease of hydrolysis of melibiose, phenyl α-galactoside and methyl α-galactoside is in the proportion, 100:123:3.3. A comparison of the action of α-galactosidases from several sources on melibiose and phenyl α-galactoside[119] is made in Table

[117] W. W. Pigman, *J. Research Natl. Bur. Standards*, *30*, 159 (1943).

[118] A. Bau, *Chem.-Ztg.*, *19*, 1873 (1895); E. Fischer and P. Lindner, *Ber.*, *28*, 3034 (1895).

[119] R. Weidenhagen and A. Renner, *Z. Ver. deut. Zucker-Ind.*, *86*, 22 (1936).

XV. In contrast with the invertase preparations from brewers' yeast, those from bakers' yeast are inactive towards melibiose and the methyl and phenyl α-galactosides;[10b] the α-galactosidase activity must be less than 10^{-4}.

In addition to invertase (β-fructofuranosidase) and the α-galactosidase of brewers' yeast, purified yeast invertase preparations contain a β-glucosidase and the otherwise unknown β-mannosidase. However, there is no evidence for the existence of a β-galactosidase, an α-n annosidase, an α-glucosidase or an α-fructofuranosidase.[10b] Phenyl β-fructopyranoside also is not appreciably hydrolyzed by yeast invertase.[120]

Yeasts are also a good source of an α-glucosidase. However, yeast α-glucosidase (maltase) as ordinarily prepared loses its activity very rapidly. For this reason yeast invertase preparations contain little if any of this enzyme, and to obtain it very mild autolysis conditions must be employed. Dried yeasts seem to be a particularly good source.[121] Such preparations

TABLE XV

Comparison of Ease of Enzymic Hydrolysis of Phenyl α Galactoside and Melibiose by α-Galactosidases from Several Sources

Source of Enzyme	Relative Ease of Hydrolysis of Melibiose Compared to Phenyl α galactoside
Bottom yeast	0 67
Sweet almond	1.1
Bitter almond	0 8
Barley malt	0 15
Aspergillus oryzae	<0 1

contain considerable quantities of invertase, but if the low temperature autolysate is treated with limited portions of activated aluminum hydroxide, the α-glucosidase is preferentially adsorbed and then may be eluted with solutions of secondary phosphates.[122]

Although the yeast invertase exerts its optimal activity at about pH 4 to 5, the yeast α-glucosidase optimum is between 6 and 7, and the activity decreases very rapidly outside of these limits.[122] Thus, at the optimum for invertase action, the α-glucosidase is practically inactive. However, at pH 6.5, there is appreciable invertase action.

It is of interest that α-glucosidase, purified by adsorption and elution, at pH 6.5 is able to hydrolyze sucrose at even a greater rate than maltose. The enzyme might be classified as an invertase as it inverts sucrose, but

[120] R. Weidenhagen, Z. Ver deut Zucker-Ind , 82, 921 (1932).
[121] R. Willstatter and E Bamann, Z. physiol. Chem , 151, 242, 273, (1926); J. R. Knieble, F L Skau and L W Lovering, J Am Chem Soc , 49, 1728 (1927)
[122] R Weidenhagen, Ergeb Enzymforsch , 2, 90 (1933)

this term is reserved for β-fructofuranosidases. The hydrolytic action[122] of α-glucosidase on sucrose (1-(β-fructofuranosyl)-α-glucopyranose) is ascribed to the presence of an α-glucosidic linkage in the sugar.

Enzymes which hydrolyze maltose and α-glucosides are widely distributed in plant and animal products and microorganisms.[123] The products which have been studied are crude and often only slightly active mixtures of various enzymes. Such preparations have been utilized for testing the action of α-glucosidase on sucrose. Since they may contain invertases as well as α-glucosidases and since the activities of these emulsins are usually low, the results have but little significance. Although sucrose is hydrolyzed by many such preparations, it is uncertain whether this is due to an invertase or an α-glucosidase component or both.

TABLE XVI

Comparison of the Relative Ease of Hydrolysis of Several α and β-Glucosides by Yeast α Glucosidase and Almond β-Glucosidase

	Aglycon Group				
	CH₃	4 Glucose	Phenyl	Saligenin	o-Cresyl
Yeast α-glucosidase	1	2 3	12	(2.4)	11.7
Almond β glucosidase	1	2 1	5.2	26	60

As previously mentioned, substitution of a group in the ortho position of phenyl β-glucoside usually produces a marked increase in ease of hydrolysis of the glucoside by almond β-glucosidase. Although the specificity of yeast α-glucosidase has not received much study, it appears that the hydrolysis of phenyl α-glucosides is not greatly affected by ortho substitution. In Table XVI, the relative ease of enzymic hydrolysis of several alpha and beta glucosides with the same aglycon groups are compared.[124]

In spite of the importance of the α-glucosidases in relation to their action on the products of diastatic hydrolysis of starches, this group of enzymes has not received much study, and they would seem to provide a desirable and important field of investigation

8. Enzymic Synthesis of Glycosides

The hydrolysis and synthesis of a glycoside may be represented by the equation:

[122] See H Pringsheim and F. Loew, *Z physiol. Chem.*, *207*, 241 (1932), R. Weidenhagen, *Z. Ver. deut. Zucker-Ind*, *78*, 788 (1928); S Hestrin, *Enzymologia*, *8*, 193 (1940); K Aizawa, *J. Biochem.* (*Japan*), *30*, 89 (1939).

[124] B. Helferich, U. Lampert and G. Sparmberg, *Ber.*, *67*, 1808 (1934).

$$ROG + H_2O \rightleftharpoons ROH + GOH \qquad \begin{array}{l} R = \text{alkyl group} \\ G = \text{glycosyl group} \end{array}$$

The equilibrium constant, K, is given by:

$$K = \frac{(ROH)(GOH)}{(ROG)(H_2O)}$$

Obviously, the presence of a high concentration of alcohol facilitates the synthesis and high concentration of water the hydrolysis. In the Fischer method for the synthesis of the glycosides (Chapter V), the catalyst is an acid, but as shown by the excellent work of Bourquelot, Hérissey and Bridel, enzymes also may be employed with some advantages over the use of acids. When acids are used, all the various isomers are formed, and the equilibrium is very complex although certain constituents predominate under the equilibrium conditions. The enzymes are very much more selective in their catalysis. Almond emulsin catalyzes the synthesis and hydrolysis of β-glucosides because of the β-glucosidase present, but no enzyme is present for the synthesis of α-glucosides (α-glucosidase). On the other hand, some yeast emulsins (yeast α-glucosidase preparations) contain an active α-glucosidase and practically no β-glucosidase. As a result almond emulsin, as shown by the excellent work of Bourquelot, may be used for the selective synthesis of β-glucosides and yeast α-glucosidase for the α-glucosides. By this means, the following β-glucosides, among others, were obtained in crystalline condition, in some instances for the first time: ethyl, geranyl, cinnamyl, butyl, hexyl, 2-ethoxyethyl, mannitol, allyl, propyl, and glucose β-D-glucosides.[125] Many α-glucosides, including the methyl, mannitol and 1,2-propanediol α-glucosides,[126] have been obtained by employment of yeast α-glucosidase as a catalyst.

For the synthesis of β-glucosides, the reactive form of glucose is β-glucose; the concentration of this form determines the reaction rate and the position of the equilibrium.[127] The equilibrium constants for the synthesis of several glucosides are as follows:

β-Glucoside	Av. K
Methyl	0.149
2-Ethoxyethyl	0.378
Mannitol	0.051

[125] E. Bourquelot and M. Bridel, *Ann. chim. phys.*, [8] **29**, 145 (1913); B Helferich and C. Lampert, *Ber.*, **68**, 2050 (1935); I. Vintilescu, C. Ionescu and A. Kizyk, *Bul. soc chim. România*, **16**, 151 (1934); I. Vintilescu, C. Ionescu and M Solomon, *ibid.*, **17**, 267 (1935)

[126] E. Bourquelot, *Ann chim.*, **3**, 287 (1915); I. Vintilescu, C. Ionescu and A. Kizyk, *Bul. soc. chim. România*, **17**, 131 (1935).

[127] I. Vintilescu, C. Ionescu and A. Kizyk, *Bul. soc. chim. România*, **17**, 137 (1935); *Ber.*, **67**, 990 (1934).

The specific action of the enzymes is advantageous in many applications, but it is also a limitation to their general use. Only a few types of glycosides can be synthesized by this method (glucoside, mannoside, and galactoside types) because of the limited number of glycosidases which occur in nature. These biological catalysts are also heat sensitive and insoluble in organic solvents. In special instances, however, their application is very advantageous. Thus, selective hydrolysis has been used to separate mixtures of α- and β-glucosides and of methyl fructosides,[125] and the enzymic synthesis of glucosides and galactosides has been made the basis of a method for the determination of these sugars in plant extracts.[126]

9. In Vivo Synthesis of Glycosides

An interesting group of experiments has been reported by L. P. Miller who has demonstrated that glucosides are synthesized in many plants when the aglycon is supplied in the nutrient solution or as a vapor in contact with the plant. Potato tubers, gladiolus corms and wheat synthesize 2-chloroethyl β-glucoside when exposed to ethylene chlorohydrin.[130] Although gentiobiose is not known to occur in gladiolus corms or tomatoes, the presence of o-chlorophenol induces the formation of the o-chlorophenyl β-gentiobioside by these plants. In the presence of both o-chlorophenol and ethylene chlorohydrin, both 2-chloroethyl β-glucoside and o-chlorophenyl β-gentiobioside are synthesized simultaneously by gladiolus corms.[131] Bottle gourd (*Lagenaria leucantha*), radish, corn, tobacco and dandelion plants form 2,2,2-trichloroethyl β-D-glucoside from added chloral hydrate.[132] The glycosides are separated as their crystalline acetates from the juices of the plants after the treatment. Previously, similar results had been obtained by Ciamician and Ravenna[133] who inoculated maize and other plants with phenols and separated the corresponding glucosides from the plants. Thus, the injection of saligenin resulted in the synthesis of salicin.

Many phenols and alcohols when injected into animals are detoxified by conjugation as glucuronides, the glycosides of glucuronic acid. Phenol-phthalein, cinnamic acid and sulfapyridine when injected into rabbits may be recovered from the urine as the corresponding glucuronides.[134] This

[125] E. F. and K. F. Armstrong, "The Carbohydrates," p. 21; Longmans, Green and Company, New York (1931); C B Purves and C S Hudson. *J Am Chem. Soc* **56**, 702, 708, 1969, 1972 (1934).

[126] M. Bridel and J Charpentier, *J pharm chim.* [7] **30**, 33 (1921)

[130] L. P. Miller, *Contrib. Boyce Thompson Inst.*, **12**, 25 (1941)

[131] L. P. Miller, *Contrib. Boyce Thompson Inst.*, **12**, 163 (1941)

[132] L. P. Miller, *Contrib. Boyce Thompson Inst.*, **12**, 167 (1941); **12**, 359, 465 (1942); **13**, 185 (1943).

[133] G. Ciamician and C. Ravenna, *Atti R. Acad. Lincei*, **25**, 3 (1916).

[134] R. T. Williams, *Biochem. J.*, **34**, 272 (1940); A. Di Somma, *J. Biol. Chem.*, **135**, 277 (1940); I. Snapper, T. F. Yü, and Y. T. Chiang, *Proc. Soc. Exptl. Biol Med*, **44**, 30 (1940); J. Soudi, *Science*, **91**, 486 (1940)

procedure is suggested for the preparation of glucuronic acid. Since *d*- and *l*-menthol and *d*- and *l*-isomenthol are conjugated under the above conditions to different extents (the formation of the *d*-menthol and *d*-isomenthol glucuronides being greater), the biological method may be utilized for the resolution of the inactive materials.[125]

10. Bourquelot Biochemical Determination of Glycosides and Oligosaccharides in Plant Materials[126]

An interesting method has been used extensively by Bourquelot, Bridel, Hérissey and associates for the analysis of glycosides and oligosaccharides in plant materials. The method depends upon the selective action of enzyme preparations from various sources in hydrolyzing certain of the components of plant extracts. Yeast emulsin (invertase) is usually used first and is followed by almond emulsin. Enzyme preparations from other sources such as *Rhamnus cathartica* emulsin (also called rhamnodiastase) and mustard seed emulsin have special applications. The yeast emulsin (invertase) hydrolyzes sucrose and oligosaccharides such as gentianose, raffinose and stachyose which have sucrose linkages in the molecule. Almond emulsin acts on β-glucosides, β-galactosides, α-mannosides and to lesser extent on α-galactosides. Emulsins prepared from plants of the *Rhamnus* type apparently contain disaccharidases which hydrolyze glycosides of primverose (6-glucose β-D-xyloside) and rutinose (6-glucose β-l-rhamnoside) into disaccharide and aglycon. Those from the mustard seeds have special enzymes hydrolyzing thioglycosides. Hydrolysis resulting from each of the treatments is shown by measurements of the reducing power and of the optical rotation before and after each treatment. These results are expressed quantitatively as the "index of enzymic reduction" which is the amount of reducing sugar formed per degree of rotation change (reducing sugar in grams/change of rotation produced by the enzyme) under specified conditions. This index differs for the various glycosides and compound sugars and may be used for the qualitative and quantitative estimation of the substances present in the plant extracts when too many are not present. The existence of many glycosides and oligosaccharides have been demonstrated by this method, many for the first time, and many plant species have been investigated. In 1920, Bourquelot summarized the results of the application of the method to 281 species of phanerogams and reported that 205 of these species contained glycosides hydrolyzable by almond emulsin. All were reported to contain sucrose, and raffinose and stachyose were common constituents.

[125] R. T. Williams, *Biochem. J.*, *34*, 48, 690 (1940).
[126] E. Bourquelot, *Compt rend*, *171*, 423 (1920); M. Bridel and C. Charaux, *Pharm. Acta Helv*, *1*, 107 (1926); C. Béguin, *ibid.*, *1*, 65, 90 (1926)

In carrying out the hydrolysis in alcoholic solution it was observed that the hydrolysis was incomplete and this led to the discovery of the synthesizing action of enzymes mentioned above. The enzymic synthesis then was utilized in methods for the biochemical determination of glucose and galactose by converting them to the corresponding glycosides.

In its present stage of development, the biochemical method probably leaves much to be desired, but additional study should make it a valuable tool for the analysis of the complex mixtures occurring in plant extracts. Methods[137] for the estimation of raffinose in the presence of sucrose have been described which depend on selective enzymic hydrolysis.

[137] C S Hudson and T S Harding, *J Am Chem. Soc*, **37**, 2193 (1915); H. S Paine and R T Balch, *Ind Eng Chem*, **17**, 240 (1925)

CLASSIFICATION AND DETERMINATION OF STRUCTURE OF THE POLYSACCHARIDES

The polysaccharides are a group of substances widely distributed in the plant kingdom but also found in the animal kingdom. They serve as reserve substances for the metabolic needs of living organisms and as structural materials in plants. They are sometimes defined as materials which on complete hydrolysis yield two or more molecules of monosaccharides. However, it is difficult to give an accurate definition because the properties usually associated with a polysaccharide gradually revert to those of the simple sugars as the molecular weight decreases. In a preceding chapter (Chapter X), the simpler members of a series of polymerized sugars were defined as oligosaccharides, in conformity with the suggestion of Helferich. In agreement with the previously given definition for the oligosaccharides, the polysaccharides might be defined as polymerized sugars with glycosidic intermolecular linkages and with more than nine monosaccharide residues in each molecule. However, as more intermediate substances become known, it may be necessary to alter this lower limit.

The most important polysaccharides are cellulose, starch and glycogen. Cellulose and starch have received a great deal of investigation, and at least the main details of the structures seem firmly established. Because of the amount of material available for starch and cellulose, they are considered separately in the following chapters. Although many of the other polysaccharides are of appreciable industrial and biological importance, much less is known about them. They are considered in Chapter XV.

The most common sugar constituent of polysaccharides is D-glucose. However, D-mannose, D- and L-galactose, D-xylose, L-arabinose, and three uronic acids (glucuronic, galacturonic, and mannuronic) and glucosamine are found among the hydrolysis products of polysaccharides. In cellulose and starch, one type of monosaccharide residue (D-glucose) is present. But in other products, notably in the plant gums and hemicelluloses, a number of different types of monosaccharide residues are present in the individual molecules.

A variety of structures occur in the various polysaccharides. Some appear to be straight-chain polymers; others are highly branched. The intramolecular linkages frequently are of the cellulose and starch type, 1,4', but other types often are encountered. Thus, yeast mannan has 1,2' glycosidic linkages; inulin has 2,1'; yeast dextran has 1,3'; alginic acid has 1,4';

peanut araban has 1,5′; and a bacterial dextran has 1,6′ linkages. Pyranoside rings are most commonly encountered, but furanoside rings have been described (inulin and arabans).

Although many of the polysaccharides have trivial or phytochemical names based on their source, such as cellulose (from cellula, L., diminutive for cell) or inulin (polysaccharide from plants of species of *Inula*), a more systematic procedure involves the replacement of the "ose" in the name of the corresponding monosaccharide by "an." Thus, polymers of mannose are mannans and of xylose are xylans. Polymers of glucose might be designated glucans, but the more common term is dextrans. The nomenclature of the polysaccharides remains in a chaotic condition, but until the structures have received additional investigation, systematism is difficult to achieve.

The classification presented below makes its primary separation on the number of monosaccharide types present in the molecule. Members of the first class (homopolysaccharides) give only one monosaccharide type when completely hydrolyzed. This class is subdivided according to the type of monosaccharide present. The second class (heteropolysaccharides), which consists of polysaccharides giving after hydrolysis more than one monosaccharide type, is subdivided according to the general source of the material because at present very few structural generalizations can be made. A second classification, also given, is based on biological function.

1. Structural Classification of the Polysaccharides

(1) Homopolysaccharides derived from single monosaccharides.
  a. Glucose polymers:
    Cellulose, lichenin, starch, glycogen, bacterial and yeast dextrans.
  b. Fructose polymers:
    Inulins, levans.
  c. Galacturonic acid polymers:
    Pectins of fruits, berries, sugar beets, etc.
  d. Polymers of mannose, galactose, xylose, ɪ-arabinose, mannuronic acid:
    Mannans, galactans, xylans, arabans, alginic acid.
  e. Glucosamine polymers:
    Chitin of crustacea, fungi and insects.
(2) Heteropolysaccharides derived from several monosaccharide types.
  a. Hemicelluloses and cell-wall polysaccharides.
  b. Mucilages, gums and gel-forming substances.
  c. Polysaccharides associated with proteins and/or microorganisms:
    Mucopolysaccharides and glycoproteins.

2. Classification Based on Biological Function

(1) Structural polysaccharides:
   Cellulose, cellulosans and pectins of plants.
   Chitin of crustacea, insects and fungi.
(2) Reserve polysaccharides:
   Starch, glycogen, inulin, lichenin and ivory nut mannan.
(3) Polysaccharides with unknown functions:
   Plant gums (possibly of pathological origin).
   Mucilages.
   Bacterial and fungal polysaccharides (many exhibit immunological reactions).

3. General Procedures for the Determination of the Structures of the Polysaccharides[1]

The first step in the determination of the structure of a polysaccharide is the purification of the product. Unfortunately, this process is one of the most difficult procedures. Cellulose, particularly in cotton and other fibers, probably is the purest naturally occurring polysaccharide. In polysaccharide mixtures such as wood and plant gums, the separation of the constituents usually is based on a fractional solution of the components and their fractional precipitation from solution. The efficiency of fractional precipitation, however, depends greatly on the choice of solvent and precipitants, and with some combinations no separation will result.[2] For cuprammonium solutions of cellulose, acetone precipitates fractions of successively lower average molecular weight, whereas sodium potassium tartrate produces no separation. Fractionation efficiency also depends upon the solute concentration, but below 2% concentration the effect seems to be negligible. In many cases, it can be questioned whether the methods in use give adequate separation of the components, and there is considerable need for improved methods for the isolation of pure polysaccharide components under conditions such that no degradation takes place.

When purified, the polysaccharides may be hydrolyzed to the component monosaccharides by the action of acids. If only a single monosaccharide is produced by the hydrolysis, the identification and analysis are not difficult. When several different monosaccharide types are produced, the identification and quantitative determination are much more difficult problems. Improved qualitative and quantitative methods for the estimation of the components of sugar mixtures are needed badly. When the molecule con-

[1] E. Anderson and L Sands, *Advances in Carbohydrate Chem.*, **1**, 329 (1945).

[2] D. R. Morey and J. W Tamblyn, *J. Phys. Chem.*, **50**, 12 (1946); O. A. Battista and W. A Sisson, *J Am Chem Soc*, **68** 915 (1946)

tains uronic acids, care must be taken that the acids are not destroyed during the hydrolysis process. For a discussion of the identification of the sugars, see Chapter III.

Valuable information often may be obtained by a study of the products of partial acid or enzymic hydrolysis. The identification of disaccharides in such mixtures provides direct evidence for the nature of the glycosidic linkage between the component residues of the polysaccharide. For example, cellobiose is a product of the partial acid hydrolysis of cellulose. It may be assumed then that the cellobiose type of linkage (β 1,4') occurs in cellulose. Similarly, the isolation of maltose from the products formed by the action of enzymes on starches provides evidence for the α 1,4' linkages in starches. However, caution must be exercised; true hydrolysis products must be distinguished from products synthesized by the action of the acids or enzymes. Thus, fructose anhydrides formed by the action of acids on inulin and the Schardinger dextrins formed by the action of certain enzymes on starches are known to be secondary, reversion products of the hydrolysis.

Certain of the polymeric linkages may be less resistant to hydrolysis than others. A study of the sugars formed in the initial stages of the hydrolysis of heteropolysaccharides may provide information concerning these linkages and the positions of certain of the monosaccharide units. Thus, during the mild hydrolysis of the water-soluble arabo-galactan from larch wood, the first residues to be eliminated are L-arabinose units, which must be in terminal positions in the molecule.

An interesting relationship between optical rotatory power and structure has been demonstrated for glucosides and glucose polymers by Reeves.[1] This relationship may have an application in structural studies. Although the specific rotations of the four 2, 3, 4 and 6-monomethyl derivatives of methyl β-glucoside are similar in value (see Table I), the rotations of the same compounds in cuprammonium solution are markedly affected by the position of the methyl group. In the cuprammonium solution, the 2-methyl and the 6-methyl derivatives are much more dextro-rotatory, and the 4-methyl derivative is much more levo-rotatory than when in aqueous solution. The 3-methyl derivative shows only a slight difference in rotation in the two solvents. Cellulose, starch and glycogen, with 1,4' linkages, have optical rotations resembling that of the methyl 4-methyl-β-glucoside. Laminarin behaves like the methyl 3-methyl-β-glucoside; this correlation confirms the chemical evidence for a 1,3' linkage for this polysaccharide (see Chapt. XV).

A. End Group Analysis. A chain polysaccharide molecule has two end monosaccharide residues which differ from the intermediate monosac-

[1] R. E. Reeves, *J. Biol. Chem.*, **154**, 49 (1944)

charide residues in having an extra unsubstituted hydroxyl group or a reducing group. For the intermediate monosaccharide residues, these groups are blocked by the connecting linkages. Methods have been devised for the determination of these "end groups." Although not of great importance for the original purpose of determining molecular weights, they are extremely valuable for measuring the extent of branching in the molecule. The amount of "end groups" in a long chain polysaccharide is too small to give an accurate estimate of the molecular weight. However, when there is an appreciable amount of branching, measurement of molecular weights by physical-chemical methods and of "end groups" by chemical methods provides an estimate of the number and average length of the side chains. The following hypothetical example illustrates this application. The number

TABLE I

Optical Rotations in Water and Cuprammonium Solution

Substance	$[\alpha]_D^{25}$	
	H₂O	Cuprammonium
Methyl 2 methyl β-glucoside	−60	+985
" 3- " β- "	−46	−86
" 4- " -β- "	−36	−1008
" 6 " -β- "	−48	+161
Cellulose	−46ᵃ	−1200
Soluble starch	+375	−715
Glycogen	+366	− 597
Laminarin	−29	+34

ᵃ In 1:1 mixture of water and Triton B

of glucose residues in a polysaccharide as given by the osmotic pressure method is 200. The number of groups which terminate chains is found to be 20 for each two hundred glucose units (10% tetramethylglucose). These results indicate the existence of 20 chains in the molecule each averaging 10 residues in length.

A large number of polysaccharides have been investigated in this fashion In succeeding chapters the results are outlined. It should be remembered that in the present stage of development in this field, the structure of polysaccharides as given in the later chapters must be considered as provisional only. Even if the results reported for the various polysaccharides are substantiated by further work, it is not possible to write definite formulas because of the various possible structures which may be written for each of the compounds. Further progress in this field requires the development of more accurate methods for the determination of molecular weights and of end groups It is particularly important to find methods for distinguishing between single and multiple branched structures; at the present, it is not possible to distinguish between single branching and multiple branching

Aldoses may be determined quantitatively by oxidation with iodine in alkaline solution. The method was applied by Bergmann and Machemer[4] to cellulose and was assumed to measure the number of reducing (hemiacetal) groups. Later workers have criticized the method because the iodine consumption increases with the time of reaction and, hence, is not stoichiometric. A critical study[5] of the reaction of iodine with cellulose and partially hydrolyzed cellulose has shown that several reactions are involved and that the primary reaction involves the oxidation of the reducing groups (hemiacetal groups) to carboxyl groups. As the effects of the slow secondary oxidation can be determined, the method may be used to estimate terminal reducing end groups. The results obtained for various partially hydrolyzed celluloses agree well with those obtained by the viscometric procedure. Cotton cellulose exhibits no oxidation under these conditions when corrections are made for secondary reactions.

For HIO_4-oxidized cellulose, and probably other oxycelluloses, the alkaline iodine methods are not applicable because of the instability of the original products to alkali[6] (see also under Oxycelluloses). Starch dextrins from commercial corn sirup give chain lengths by this method agreeing with the number of carboxyl groups produced as measured by titration and by analysis of the potassium salts.[7]

A common method for the testing of cellulose products is the determination of the so-called "copper number," originally suggested by Schwalbe.[8] The "copper number" is determined by heating the product with an alkaline copper salt solution and measuring the copper oxide formed. As a qualitative test, it may have some value, but the results are difficult to interpret since the reaction is not stoichiometric and since celluloses are oxidized by air in alkaline solution.

A somewhat similar empirical test, useful for characterizing polysaccharides, is the alkali-lability determination used for starch products.[9] The alkali-lability is defined as the number of cc. of 0.1 N sodium hydroxide consumed by 1 g. of starch during digestion in the alkali for 1 hour at 100°C. The results are an empirical expression of the acids produced from the terminal reducing group by the action of the alkali. The method provides a sensitive expression of the degree of degradation in a given polymeric series

[4] M Bergmann and H Machemer, *Ber* , *63*, 316, 2304 (1930)

[5] A R Martin, L Smith, R L Whistler and M. Harris, *J Research Natl. Bur. Standards*, *27*, 449 (1941); H A. Rutherford, F W Minor, A R Martin and M Harris, *ibid.*, *29*, 131 (1942)

[6] E. Pacsu, *Textile Research J* , *16*, 105` (1946).

[7] M Levine, J. F. Foster and R M. Hixon, *J Am. Chem. Soc.*, *64*, 2331 (1942)

[8] See D A Clibbens and A Geake, *J Textile Inst* , *15*, T27 (1924); J O Burton and R H Rasch, *Bur Standards J Research*, *6*, 603 (1931).

[9] T J. Schoch and C C Jensen, *Ind Eng Chem* , *Anal Ed* , *12*, 531 (1940)

The carboxyl groups produced by the oxidation of aldehyde groups with Fehling solution provide another method for the estimation of end groups. Methylene blue- and base-binding capacity have been used for the measurement of the number of carboxyl groups.[10]

Hemiacetal groups react with amines and hydrazines (see Chapter IX). For cellulose, the N-content of the phenylhydrazine derivative is a measure of the number of end groups.[11] Also, the nitrogen atoms introduced in this manner can be used to fix acid dyestuff ions which subsequently can be removed and determined by colorimetric methods. The latter procedure is said to be quantitative and to give results with hydrocelluloses that agree with molecular weight determinations made by physical methods (osmometric, etc.).[12] For starches, adsorption of the reagent occurs and invalidates the method.[7,13]

Periodic acid oxidation of polysaccharides having 1,4′ linkages between hexose units should yield one mole of formic acid per non-reducing terminal residue and two per terminal reducing group (see p. 328 and 436). For 1,6′-linked polysaccharides each residue linked in this manner also gives one mole of formic acid. The quantities of formic acid produced from various polysaccharides are in fair agreement with determinations by other methods.[14] For most starches there is one end group per 20 glucose residues. Cellulose shows at least 1000 units per chain, and inulin has approximately 25 fructose units per chain. The amount of formaldehyde formed also has been used[15] for studies of chain lengths.

The concurrent hydrolysis and mercaptylation of celluloses and starches has been used by Wolfrom for the estimation of the molecular weights of these substances.[16] The reaction takes place in concentrated hydrochloric acid solution in the presence of ethyl mercaptan. Ethyl mercaptan reacts with the reducing hemiacetal groups of sugars and polymers with the formation of mercaptals (see under Mercaptals). The sulfur content at various times after the commencement of the reaction is measured and used to calculate the number of chain "ends." Extrapolation of the results to zero time gives the number of "end groups" in the original substance. The values

[10] E. Husemann and O. H. Weber, *Naturwissenschaften*, *30*, 280 (1942). A. M. Sookne, H. A. Rutherford, H. Mark and M. Harris, *J. Research Natl. Bur. Standards*, *29*, 123 (1912)

[11] See. C. J. Staud and H. LeB. Gray, *Ind. Eng. Chem., Anal. Ed.*, *1*, 80 (1929)

[12] E. Geiger and A. Wissler, *Helv. Chim. Acta*, *28*, 1638 (1945)

[13] F. Muller, *Helv. Chim. Acta*, *29*, 21 (1946).

[14] F. Brown, S. Dunstan, T. G. Halsall, E. L. Hirst and J. K. N. Jones, *Nature*, *156*, 785 (1945).

[15] C. J. Caldwell and R. M. Hixon, *J. Biol. Chem.*, *123*, 595 (1938).

[16] M. L. Wolfrom and J. C. Sowden, *J. Am. Chem. Soc.*, *60*, 3009 (1938); M. L. Wolfrom, D. R. Myers and E. N. Lassettre, *ibid.*, *61*, 2172 (1939).

obtained in this manner seem to be smaller than those obtained from viscosity measurements.

One end group in a chain of condensed monosaccharide units contains one more hydroxyl group than the preceding units. It was shown by Haworth and Machemer[17] that methylation of glucose polymers followed by hydrolysis gives trimethylglucose accompanied by a small amount of tetramethylglucose. The proportion of the two methyl sugars provides a measure of the chain length of the polysaccharide. The method has been widely employed, but, in many instances, the results were not in agreement with those by the physical-chemical methods, in that much smaller molecular weights were obtained by the end group method. These discrepancies have been reconciled by the realization that branched chains may be present in the polysaccharide molecule.[18] The terminal group of each branched chain provides a molecule of tetramethylglucose. Particularly for cellulose, the methylation must be carried out in an inert atmosphere to prevent degradative oxidation. A more recent extension of the method allows the determination of the number of monosaccharide units which carry side chains. For a glucose polymer, these would necessarily appear as dimethylglucoses if only one side chain were attached to a main chain unit; for such a condition, the quantity of dimethylglucose must be equal to that of the tetramethylglucose.

B. Structure of a Bacterial Dextran (an Example of Structural Analysis). As an example, the results[19] obtained from the structural studies on the dextran synthesized by the action of *Leuconostoc mesenteroides* on sucrose will be cited in detail. The methylation of the dextran is carried out first with sodium hydroxide and dimethyl sulfate in an atmosphere of nitrogen and completed in liquid ammonia solution using sodium and methyl iodide. The methoxyl content of the final product agrees with the theoretical value of 45.6 per cent. By treatment of the methylated dextran with hydrogen chloride in methyl alcohol at 140°C., the linkages between the glucose units are broken, and a mixture of methylated methyl glucosides are obtained. These compounds are separated into fractions by fractional distillation in a modified Podbielniak column. The results are tabulated in Table II.

The isolation of the components of mixtures of methylated sugars usually has been done by fractional distillation methods.[20] Since trimethylglucose is most soluble in water and tetramethylglucose in chloroform, a preliminary partition of a mixture of two compounds between chloroform and water may increase the efficiency of the

[17] W. N. Haworth and H. Machemer, *J. Chem. Soc.*, 2270 (1932)

[18] K. Hess and F. Neumann, *Ber.*, 70, 728 (1937), W. N. Haworth, R. E. Montonna and S. Peat, *J. Chem. Soc.*, 1899 (1939)

[19] I. Levi, W. L. Hawkins and H. Hibbert, *J. Am. Chem. Soc.*, 64, 1959 (1942)

[20] See, for example E. L. Hirst and G. T. Young, *J. Chem. Soc.*, 1247 (1938)

separation [21] Adsorption on silica gel or alumina followed by fractional elution pro vides a means of separating di , tri and tetra methylglucoses or their aroyl esters [22] This method appears particularly promising

The theoretical methoxyl contents of methyl di-, tri- and tetra-methylglucosides are, respectively, 41.9, 52.6 and 62.0 per cent. Fractions 1, 3, 4 and 6 are then essentially pure, but the much smaller fractions 2 and 5 are mixtures for which the composition is calculated from the methoxyl analysis. The nature of the fractions is shown by conversion to crystalline derivatives of known properties. The approximate molar ratios of the three products is found to be: methyl 2,3-dimethylglucoside, 1; methyl 2,3,6-trimethylglucoside, 3; and methyl 2,3,4,6-tetramethylglucoside, 1. Although these results do not lead to an unequivocal structure, they fix the

TABLE II

Fractionation of Methylated Methyl glucosides Obtained by Methanolysis of Methylated Dextran

Fraction No	Weight of Fraction	%OCH₃	"Tetra" Content	"Tri" Content	"Di" Content
	g		g	g	
1	1 201	61 0	1 201		
2	0 207	57 8	0 113	0 094	
3	0 605	52 5		0 605	
4	2 511	52 4		2 511	
5	0 397	46 5		0 172	0 225
6	1 013	42 0			1 013
Total			1 317	3 682	1 238
Ratio ("tri" content assumed as 3 00)			1 07	3 00	1 01

general pattern. As the basic repeating unit is composed of five glucose residues, the side chains cannot be longer than four glucose units. If the side chains have four units, the main chain consists of glucose residues each of which carries a side chain (Formula I) and which are responsible for the dimethylglucose produced. The side chains cannot be shorter than the one glucose unit which yields the tetramethylglucose found in the analytical procedure; in this limiting case, the units with three unsubstituted hydroxyls must lie in the main chain between those carrying the side chains (Formula II). It is not possible at present to distinguish between these possibilities or any intermediate type of structure, e.g., two units in the side

[21] J Y Macdonald J Am Chem Soc , 57, 771 (1935)

[22] E J Norberg I Auerbach and R M Hixon, J Am Chem Soc 67, 342 (1945), J K N Jones J Chem Soc , 333 (1944), D J Bell, ibid , 473 (1944), J K Mertz walker D M Carney and F T Fowler J Am Chem Soc 65 2507 (1943)

chains and one between the glucose residues in the main chain to which the side chains are attached.

```
       1-4'                    1'-4
   -------glucopyranose-------
            |6'
            |1
        (glucopyranose)₄
            (I)
```

```
   1'-4              1-6'              1'-6              1-4'
- ----glucopyranose---(---glucopyranose---)₂---glucopyranose---- -
          |6
          |1'
      glucopyranose
```

(II)
(Two possible repeating units)
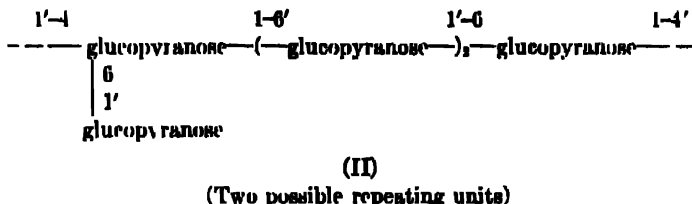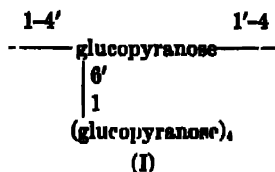
4. General Procedures for the Determination of the Molecular Weight of High Polymers[23]

One of the most important and most difficult of the problems concerned with the elucidation of the structures of the polysaccharides is the determination of the molecular size. Considerable progress has been made in this direction, but there still remain many points of disagreement particularly for the less studied polysaccharides. The problem is complicated by association of molecules to form "supermolecular" aggregates, but aggregation can be overcome in most instances by extrapolation of the observed results to infinite dilution where association is at a minimum. Kraemer[24] has provided evidence showing that for cellulose true molecular weights are measured under the proper conditions. Thus, corresponding molecular weights are obtained for a cellulose, the partially and completely acetylated products derived from it and the regenerated cellulose obtained by deacetylation. The same conclusion is drawn from the identity of the molecular weights of a cellulose acetate in various solvents.

Another difficulty arises from the inhomogeneity of many polysaccharide products, and many reported results are of doubtful significance. Physical-chemical methods based on counting the molecules (osmotic pressure, freezing point depression, etc.) and purely chemical methods give "number-average" molecular weights which are affected principally by the smaller molecules present. On the other hand, the viscosity method gives a "weight-

[23] K. H. Meyer, "High Polymers," Vol 4, p 12, Interscience Publishers (1942); M. Samec, *Kolloid-Beihefte*, **51**, 37b (1940); C. B. Purves (Editor: E. Ott), "High Polymers," Vol 5 p 88; Interscience Publishers (1943); H. M Spurlin, *ibid.*, Vol 5 p 910 (1943).

[24] E. O. Kraemer, *Ind. Eng. Chem.*, **30**, 1200 (1938).

average" molecular weight mainly dependent on the larger molecules. Both types and still a third type, "Z-average" molecular weight, are obtained from ultracentrifugal data. Lansing and Kraemer[25] define these various molecular weights as follows:

$$\text{Number average, } M_n = \frac{\sum (n_i M_i)}{\sum n_i} = \frac{\sum w_i}{\sum (w_i/M_i)}$$

$$\text{Weight average, } M_w = \frac{\sum (n_i M_i^2)}{\sum (n_i M_i)} = \frac{\sum (w_i M_i)}{\sum w_i}$$

$$\text{Z-average, } M_z = \frac{\sum (n_i M_i^3)}{\sum (n_i M_i^2)} = \frac{\sum (w_i M_i^2)}{\sum (w_i M_i)}$$

(n_i is the number of molecules of molecular weight M_i, and w_i is the total weight of the molecular species "i.")

The degree of homogeneity of high polymers requires more consideration than has sometimes been the case. No estimate of the number-average molecular weight can be made from viscometric measurements of products of unknown homogeneity. For a perfectly homogeneous polymer, the ratio M_w/M_n is unity; the ratio increases with the heterogeneity with respect to molecular size, and the value of the ratio may be taken as a measure of the heterogeneity. This ratio is an important characteristic for the evaluation of commercial products. A certain amount of polymolecularity may be desirable in some products although usually the presence of materials of extremely low molecular weight may affect the properties adversely.[26] In general the most significant measure is the number-average molecular weight (or degree of polymerization). Thus, the mechanical properties of films prepared from a series of cellulose acetate fractions and blends of fractions have been shown[27] to be related to the number-average degree of polymerization (D.P.) rather than to the weight-average D.P

A. Osmotic Pressure Method. In very dilute solutions, the osmotic pressure is directly proportional to the concentration of the solute and inversely proportional to the molecular weight. According to the van't Hoff equation:

[25] W D Lansing and E O. Kraemer, *J Am Chem Soc*, **57**, 1369 (1935) See also I Jullander, *Arkiv Kemi, Mineral Geol*, *21A*, No 8 (1945), *Chem Abst*, *40*, 264 (1946)

[26] H M Spurlin *in* "Cellulose and Cellulose Derivatives," (E Ott, Editor) p. 930, Interscience Publishers, Inc., New York (1943), A M Sookne and M Harris, *J. Research Natl Bur Standards*, **34**, 459 (1945)

[27] A M Sookne and M Harris, *J Research Natl Bur Standards*, **34**, 467 (1945)

$$M = RT/(\pi/c)_{c=0} = 2.53 \times 10^{5}/(\pi/c)_{c=0} \text{ (at } T = 298°K).$$

M = molecular weight;
c = concentration of solute in grams per 100 ml.;
π = osmotic pressure in grams per cm.^{2}

This equation is strictly valid only at infinite dilution, and considerable error may be introduced by using values obtained at finite concentrations. For the extrapolation to infinite dilution, a number of equations relating osmotic pressure and concentration have been suggested.[28] The extrapolation may be made by plotting π/c vs. c and using the extrapolated value of π/c at infinite dilution, $(\pi/c)_{c=0}$, to calculate M.

Maximal molecular weights determinable by this method are of the order of 500,000; measurements on substances with greater molecular weights are made inaccurate by surface tension and temperature influences. Several types of apparatus for the measurement of osmotic pressure have been described.[29]

Results of the application of the osmotic pressure method to cellulose and starch are given in later chapters (see p. 532 and p. 570).

B. Ultracentrifuge.[30] In a gravitational field, particles and molecules distribute themselves in height according to the well known law of barometric distribution:

$$P = P_0 e^{\frac{M \cdot g(h-h_0)}{RT}}$$

M = molecular weight
g = gravitational constant
P and P_0 are the pressures at the heights h and h_0.

In the classical experiments of Perrin, this formula was utilized for the determination of Avogadro's constant (N) by measuring the distribution of microscopic particles under the influence of gravity.[31] In the ideal case,

[28] See: K. H. Meyer, *loc. cit.*[22]; A. Bartovics and H. Mark, *J. Am. Chem. Soc.*, **65**, 1901 (1943).

[29] R. O. Herzog and H. M. Spurlin, *Z. physik. Chem.*, Bodenstein Festband, 239 (1931); S. R. Carter and B. R. Record, *J. Chem. Soc.*, 660 (1939); R. E. Montonna and L. T. Jilk, *J. Phys. Chem.*, **45**, 1374 (1941); G. V. Schulz, *Z. physik. Chem.*, A176, 317 (1936); R. M. Fuoss and D. J. Mead, *J. Phys. Chem.*, **47**, 59 (1943).

[30] The Svedberg and K. Pedersen, "The Ultracentrifuge;" Oxford University Press (1940); The Svedberg, *Ind. Eng. Chem., Anal Ed.*, **10**, 113 (1938); C. O. Beckmann and Q. Landis, *J. Am. Chem. Soc.*, **61**, 1495 (1939); H. Bomke, *Naturwissenschaften*, **32**, 185, 250 (1944).

[31] In this case, P is replaced by n, the number of particles in a unit volume, and M by $N \cdot m$ where m is the mass of a single particle.

each component of a mixture distributes itself independently according to the above law. For molecules, the variation of density is so small that the method has no practical value for the determination of the molecular weights. However, the procedure can be utilized for large molecules by exposing them to centrifugal fields. High speed centrifuges and suitable procedures for this purpose have been developed. Svedberg reports that fields of 400,000 times the force of gravity may be employed and that fields of more than double this strength have been attained.

Kraemer[32] cites the following advantages of the ultracentrifuge method over other methods:

1. "It has the same thermodynamic foundation as osmotic pressure or vapor pressure methods.

2. It is accordingly not influenced by particle shape.

3. In general it is not affected by solvation.

4. Its sensitivity increases with increase in particle size.

5. It can be used with complex solvents like cuprammonium with which osmotic pressure measurements would be very difficult.

6. It avoids difficulties associated with semipermeable membranes.

7. It permits recognition of the uniformity or nonuniformity of particle size, and it can give a quantitative rating of the degree of nonuniformity.

8. For solutes containing relatively small molecular weight contaminants, it is much less adversely affected than osmotic pressure and other methods."

SEDIMENTATION EQUILIBRIUM

The method is applied by centrifuging a solution in a cell which is specially shaped to minimize convection currents and which is arranged so that light may be passed through the solution and be recorded in a direction perpendicular to the plane of rotation. After equilibrium has been established between diffusion and sedimentation due to the gravitational field, the concentration of the solute is determined at several points in the cell. This may be done by measuring the light absorption (using ultraviolet light for polysaccharides) or the refractive index. The molecular weight (M) is then calculated from the equation:

$$M = \frac{2RT \cdot \ln(c_2/c_1)}{(1 - V\rho)(x_2^2 - x_1^2)\omega^2}$$

(V is the partial specific volume of the solute; ρ is the density of the solvent; ω is the angular velocity; and c_1 and c_2 are the solute concentrations at the distances x_1 and x_2 from the rotational axis.) Information concerning the homogeneity of the solute may be obtained by calculating the molecular

[32] Quoted from E. O. Kraemer.[34]

weight for a number of values of "c" and "x." When only particles of a single size are present, the calculated values of "M" will remain constant; otherwise, variations in "M" will be observed.

SEDIMENTATION VELOCITY

A second method, which requires higher speed centrifuges, depends on measurement of the velocity of sedimentation. If the solute is homogeneous, a sharp boundary is obtained marking the upper part of the sedimentating solute. The boundary moves away from the axis of rotation, and the velocity of sedimentation may be determined by measuring its position at various time intervals. The observed velocity is divided by $\omega^2 x$ to give the *sedimentation constant* (S) which expresses the velocity for unit field strength at a given temperature and for a specified solvent. From S, the molecular weight is calculated by use of the relation:

$$M = \frac{RT \cdot S}{D(1 - V\rho)}$$

The symbols have the previously defined significance, the only new symbol being "D," the diffusion constant. This constant is evaluated by measuring the change of concentration of the solution in a stationary tube as the solute diffuses into the pure solvent with which the solution forms a liquid boundary.

If the solute consists of several kinds of molecules, each will form a separate boundary. Hence, the method may be used to measure the homogeneity of the solute and to determine the molecular weights of each of the components. The presence of many different types of molecules is indicated by the presence of a diffuse boundary. Particularly for chain polymers of the type of many of the polysaccharides, the observed molecular weight is dependent on the concentration. Therefore, it is necessary to make measurements at various concentrations and to extrapolate to infinite dilution.

Results of the application of the ultracentrifugal method to cellulose and starch are given in later chapters (see p. 532 and p. 570).

C. Viscosity Measurements. Ordinarily, the viscosity of solutions of substances of a polymeric series increases with the molecular weight of the members of the series. Staudinger, to whom much of the credit for the development of this method is due, has expressed an empirical relation connecting the observed viscosity ($\eta_{solution}$) and the molecular weight (M):

$$\eta_{sp} = K_m \cdot M \cdot c; \qquad M = \eta_{sp}/c \cdot K_m.$$

The specific viscosity, η_{sp}, is calculated from the viscosity of the solution by virtue of the relation:

$$\eta_{sp} = \frac{}{\eta_{solvent}} - 1 = \eta_{rel}$$

The concentration, c, is expressed in moles of basic structural unit per liter, i.e., moles of $C_6H_{10}O_5$ in the case of cellulose and starch. When the concentration is expressed as $g/l = (c_{gr})$, the degree of polymerization $(D.P.)$ is obtained. The degree of polymerization expresses the number of basic units in the molecule. The proportionality constant, K_m, shows fair constancy for a given polymeric series but varies for different series, solvents and temperatures. Originally K_m was evaluated by Staudinger for several lower members of a given series and was assumed to hold for the higher members of the same series, but considerable error is introduced by such a method. A better procedure involves the determination of the molecular weights of several members of the series by methods having a better theoretical basis (e.g., by measurements with the ultracentrifuge or an osmometer) and the use of these values for the evaluation of K_m from the viscosity data. Since viscometric measurements give "weight" averages and the other data other types of averages (see above), the values obtained for inhomogeneous products require care in their interpretation. The more convenient viscosity method then is employed for the determination of the molecular weights for other members of the particular polymeric series. Because of its ease of application, the viscometric method has been widely employed for the characterization of commercial cellulose products.

The relation between viscosity and molecular weight has been tested for a series of crystalline polyoxyethylene glycols; except for the lower members of the series, the Staudinger equation has been found to apply.[23] If another constant (β) is added to the equation giving it the form $\eta_{sp}/c = K \cdot M + \beta$, the relation between the viscosity and the molecular weight may be expressed over the entire range studied.

Although the Staudinger equation and its modifications were empirical in origin, theoretical equations of the same form have been derived for the specific viscosity of a dilute solution of randomly-kinked chain molecules.[24]

Although Staudinger assumed originally that in dilute solution the reduced viscosity (η_{sp}/c) is independent of the concentration, a considerable variation frequently is observed at the smallest usable concentrations. A more characteristic quantity is the "limiting viscosity" of K. Meyer which is obtained by extrapolating η_{sp} c to zero concentration. It is symbolized as:

$$\lim_{c \to 0}\left[\frac{\eta_{sp}}{c}\right] \text{ or } \left[\frac{\eta_{sp}}{c}\right]_{c=0}$$

Other methods of correcting for concentration effects are described by Meyer.[23]

[23] R. Fordyce and H. Hibbert, *J. Am. Chem Soc* , *61*, 1912 (1939). For additional discussion of the relationship between viscosity and molecular weight see: P J. Flory, *ibid* , *65*, 372 (1943); A. Bartovics and H. Mark, *ibid.*, *65*, 1901 (1943).

[24] M L Huggins, *J. Am Chem. Soc.*, *64*, 2716 (1942).

Results of the application of this method to polysaccharides are given in later chapters (Cellulose, Starch, Pectins, etc.).

D. Light Scattering Method.[35] [36] When light waves pass through a solution, the well-known Tyndall effect results from the vibration of loosely-bound electrons and the emission in all directions of radiation having the same frequency as the original beam. For particles small in comparison with the wave length of the light, the light will be scattered symmetrically

TABLE III

Molecular Weights and Dimensions of Cellulose Acetate Fractions

Fraction	Mol Weight		Distance Between Ends (Å) Assuming		
	Light scattering	Osm pressure	Rigid rod[a]	Random coil[a]	Full extension[b]
4B	173,000	163,000	1900	1340	3100
23B	135,000	133,000	1900	1340	2400
16B	77,000	75,000	1550	1120	1410
32B	60,000	65,000	1550	1120	1250
31B	52,000	–	1380	960	1000

[a] Root mean square value from dissymmetry factor
[b] Mol Wt /length of $C_6H_{10}O_5$ unit (5.1 Å)

in all directions. The resulting turbidity is related to the size of the scattering particles according to the equation:

$$H\frac{c}{\tau} = \frac{1}{M_2} + \frac{2B}{RT}c \qquad \text{where}$$

$$H = 32\,\pi^3 n^2\,\frac{(\partial n/\partial c)^2}{3\lambda N_0}$$

τ = turbidity
n = refractive index
c = concentration
λ = wave length of light used
N_0 = Avogadro's number
M_2 = molecular weight of the scattering particles (solute)
B = deviation from van't Hoff's law as expressed in the equation for osmotic pressure ($\pi = RTc/M + Bc^2$).

The above equation is linear in form ($y = b + ax$) and a plot of Hc/τ vs. c should be linear with the intercept as $1/M_2$. For the molecular weight de-

[35] P. Debye, *J Applied Phys*, **15**, 456 (1944)
[36] R S Stein and P. Doty, *J. Am Chem. Soc*, **68**, 159 (1946).

termination, measurements of the refractive index (n), of the variation of n with concentration and of the intensity of the scattered light are required.

In the derivation of the above equation, it was assumed that the size of the scattering particles is negligible compared with the wave length of the incident light. When this condition is not realized, the light scattered from one portion of a particle (molecule) will be out of phase with that from another portion and interference effects will be produced, which result in a dissymmetry in the angular distribution of the scattered light. When the angular distribution is measured and found to be dissymmetric, a correction factor can be introduced which when multiplied by the molecular weight as obtained from the use of the above formula gives a molecular weight corrected for the dissymmetry factor.

For molecules smaller than about 275 Å (1/20) of the wave length of light, e.g., 5461 Å) interferences from different portions of the same molecule will not be serious, but above this size the dissymmetry must be considered. This value would correspond to a $D.P.$ of about 50 for an extended cellulose chain (275, 5.1). Hence, many polysaccharides will give dissymmetric distributions.

Although this factor complicates the method, it also provides an independent means of calculating the dimensions and dissymmetry of molecules. The results obtained by Stein and Doty[26] on fractionated cellulose acetates are given in Table III. As shown in the table, the molecular weights calculated by this method agree with those from the osmotic pressure method. The molecules for fractions with molecular weights greater than about 60,000 are not fully extended.

E. Other Methods. Other properties which depend upon molecular weight have been employed for the determination of the molecular weights of polymers, but the osmotic, centrifugal and viscometric methods have been the most widely employed. The rate of diffusion of a solute into the pure solvent and the depression of the freezing point (cryoscopic method) have some value, particularly for substances with small molecular weights. The method of ultrafiltration through membranes of known pore size is not of great value for the chain type of polymers of the type of most of the polysaccharides. More details of these methods are given by Samec, Spurlin and Meyer.[28]

CELLULOSE[1]

1. Occurrence and Structure

A. Occurrence. The cell membranes of the higher plants are composed principally of the polysaccharide cellulose, and woody tissues may consist of more than 50 per cent of this material. The entire vegetation of the world has been estimated[2] to contain carbon equivalent to about 1000 billion kg. of carbon dioxide, and of this the major portion is found as cellulose. Although at the present time this polysaccharide is of great industrial importance, it will undoubtedly attain still greater significance in the future as a source of energy and of important chemicals when the natural reserves of oil and coal disappear.

The cell walls of practically all phanerogams contain cellulose, but the quantity present varies greatly. Young leaves may contain as little cellulose as 10 per cent of the dry weight, but in older leaves the content may reach 20 per cent. Wood and bark are considerably richer in cellulose, the former often containing more than 50 per cent, and cotton, which represents the richest source, consists of more than 90 per cent of the polysaccharide. Cellulose has been frequently reported as a constituent of lower plants, but the identification has usually been made by color tests of doubtful validity. However, on the basis of the isolation of cellobiose octaacetate after acetolysis and of material insoluble in strong alkali, cellulose has been identified in wood, Spanish moss, the rhizomes of brake fern and the capsule stalks of hair cap moss.[3] Although usually only considered a plant product, cellulose (tunicin) is also found in certain marine animals (Tunicata, e.g., *Phallusia mamillata*, *Polycarpa varians*). Iceland moss contains a similar polysaccharide, lichenin.

Isolation and Purification. Cotton comprises the raw material used for most structural studies because it is fairly pure and is easily purified by methods which probably do not cause much degradation.[4] The raw cotton

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[1] J. T. Marsh and F. C. Wood, "An Introduction to the Chemistry of Cellulose;" Van Nostrand, New York (1942); E. Ott (Editor), "High Polymers," Vol. 5; Interscience Publishers, New York (1943). K. H. Meyer, "High Polymers," Vol. 4; Interscience Publishers, New York (1942); E. Heuser, "The Chemistry of Cellulose," Wiley & Sons, New York (1944); L. E. Wise (Editor), "Wood Chemistry," A. C. S. Monograph 97; Reinhold, New York (1944); C. F. Cross, E. J. Bevan and C. Beadle, "Cellulose," Longmans, Green and Company, London (1918).

[2] H. Schroeder, *Naturwissenschaften*, **7**, 28 (1919).

[3] See: W. M. Harlow and L. E. Wise, *Am. J. Botany*, **25**, 760 (1938)

[4] A. B. Corey and H. LeB. Gray, *Ind. Eng. Chem.*, **16**, 853, 1130 (1924); R. K. Worner and R. T. Mease, *J. Research Natl. Bur. Standards*, **21**, 609 (1938).

is first subjected to a dewaxing process which involves extraction with hot alcohol. The dewaxed fiber, after a washing with water, is treated with boiling 1% sodium hydroxide solution to remove associated pectic materials. Such purified dewaxed and depectinized cotton usually contains less than 0.05% ash and about 99.8% of material (α-cellulose) insoluble in 17.5% alkali.

The occurrence of cellulose in woody tissues is of considerable commercial importance since paper and synthetic cellulose fibers are obtained primarily from this source. Wood is also becoming somewhat important as a source of cellulose for the manufacture of cellulose esters and ethers, which were formerly made largely from cotton linters. From the standpoint of structural studies, wood cellulose has less importance since its purity is doubtful, and considerable degradation takes place in the purification process. The cellulose content (α-cellulose) of woody materials is usually taken as that portion of the cell-wall polysaccharides which, in the cold, will not dissolve in 17.5% sodium hydroxide solution. The material which precipitates upon acidification of the alkaline extract is named β-cellulose and that soluble in acid solution, γ-cellulose (see also p. 621). Launer suggests a volumetric method for the determination of these constituents.[5] The alkali-soluble material consists principally of hemicelluloses, polyuronic acids and residual lignin. For industrial purposes, wood purification is accomplished by treatment with alkalies, sodium sulfide or calcium bisulfite. Straw and other materials, which contain greater quantities of lignin, are treated first with chlorine to dissolve the lignin and then with bisulfite. The purified celluloses usually are bleached by the action of chlorine followed by hypochlorite or peroxide.

B. Chemical Evidence for Structure. Fuming hydrochloric acid (40% HCl) hydrolyzes cellulose to D-glucose in a yield of 95 to 96%.[6]

As the empirical formula is $C_6H_{10}O_5$, cellulose might be either an anhydrohexose or a chain polymer formed by the elimination of a mole of water from successive pairs of glucose units. The chain would have to be sufficiently long so that the analytical results could agree with either $C_6H_{10}O_5$ or $(C_6H_{12}O_6)_n\text{-}(n-1)H_2O$. The extremely low reducing power of cellulose and the production of reducing sugar as a result of acid hydrolysis agree with both of these possible types of structure. Inasmuch as molecular weight determinations (to be discussed later) support the latter formula and completely eliminate the anhydroglucose structure, cellulose must consist of glucose residues with connections formed by the elimination of the elements of water.

[5] H. F. Launer, *J. Research Natl. Bur. Standards*, *20*, 87 (1938); *18*, 333 (1937).

[6] R. Willstätter and L. Zechmeister, *Ber.*, *46*, 2401 (1913); J. C. Irvine and E. L. Hirst, *J. Chem. Soc.*, *121*, 1585 (1922).

The presence of three unsubstituted hydroxyl groups for each glucose residue is demonstrated by the formation of triacetates, trinitrates and trimethyl ethers of the formula $(C_6H_7O_5(CO-CH_3)_3)_n$, $(C_6H_7O_5(NO_2)_3)_n$, $(C_6H_7O_5(CH_3)_3)_n$. The formation of a monotrityl derivative of cellulose[7,8] is due to the presence of a primary hydroxyl group in each glucose residue. The formation of monotosyl derivatives and the replacement of the tosyloxy groups by iodine through reaction with sodium iodide in acetone or acetonyl-acetone solution proves the existence of unsubstituted primary alcoholic groups[8,9] (see Tosyl esters, Chapter IV).

Of particular importance for showing the nature of the polymeric linkage are the acetolysis experiments. Cellulose under acetylating conditions in the presence of an acid catalyst is degraded with the formation of high yields of cellobiose octaacetate.[10] After correction for the amount of cellobiose octaacetate acetolyzed under the same conditions, the maximal yield of the disaccharide is estimated by Freudenberg as 50 to 60%. This yield compares with a value of 30% calculated on the assumption that the cleavage of the linkages is entirely a random process. On the assumption that the ease of hydrolysis increases with decrease of the degree of polymerization of the hydrolytic products and that all linkages in each degraded particle are hydrolyzed with the same ease, a theoretical yield of 67% is calculated. In a study of the yields of cellobiose octaacetate produced, Spencer[11] reported a maximum of 42.3% but, in disagreement with Freudenberg, found that the octaacetate was not appreciably degraded under the acetolysis conditions.

Since cellobiose octaacetate is not synthesized from glucose under the conditions of acetolysis, and since no other disaccharides are produced, the cellobiose type of linkage must be the major type present in the original polysaccharide. As described elsewhere (see Cellobiose), the disaccharidic bond of cellobiose lies between carbon 1 of one glucose residue and carbon 4 of the other and has a beta configuration. A chain structure with β-glucosidic linkages agrees with the studies of the optical rotatory power during hydrolysis of cellulose by strong acids and of the kinetics of the process. These studies show that cellulose has the properties predicted by the extrapolation to infinite chain length of those of the short-chain cellohexaose, cellobiose, etc., of known structure.[12]

[7] B. Helferich and H. Koester, Ber., 57, 587 (1924).

[8] W. M. Hearon, G. D. Hiatt and C. R. Fordyce, J. Am. Chem. Soc., 65, 2449 (1943).

[9] T. S. Gardner and C. B. Purves, J. Am. Chem. Soc., 64, 1539 (1942).

[10] A. P. N. Franchimont, Ber., 12, 1938 (1879); Z. H. Skraup, Monatsh, 26, 1415 (1905).

[11] C. Spencer, Cellulosechem., 10, 61 (1929).

[12] K. Freudenberg and G. Blomqvist, Ber, 68, 2070 (1935); H. Hibbert and E. G. V. Percival, J. Am. Chem. Soc., 52, 3995 (1930).

The 1,4' type of connection between the glucose components is given final substantiation by the practically quantitative yield of 2,3,6-trimethylglucose obtained from the hydrolytic products of fully methylated cellulose. The following structure illustrates a cellulose chain with the cellobiose structure as the repeating unit.

Cellulose chain
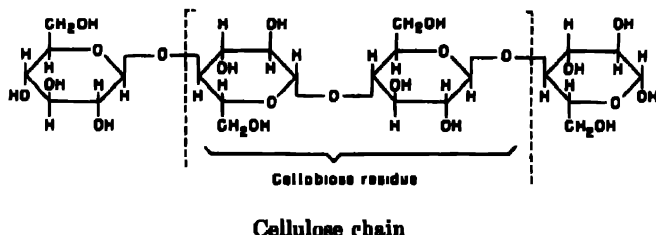
C. Molecular Weights of Cellulose and Derivatives.[14] Many moleculai weight measurements are given for celluloses and cellulose derivatives, but considerable caution must be exercised because of the many difficulties involved. The methods of measuring the moleculai weights of cellulose require the use of solutions. The number of solvents available is very limited, and degradation may take place during the dissolution. When derivatives are employed, there is often no assurance that degradation has not taken place during their preparation. It should be noted also that most celluloses, even cotton cellulose, are highly heterogeneous as far as moleculai distribution is concerned. The presence of minute quantities of oxygen may influence the results markedly.

Table I, taken from the data given by Staudinger,[14] gives the results obtained for several carefully prepared cellulose products and for some commercial cellulose products. The values were determined by the viscosity method in ammoniacal copper oxide solutions, and the molecular weights were calculated from the degree of polymerization by multiplication by the factor 162 (mol. wt. of $C_6H_{10}O_5$).

Similar studies have been carried out by the ultracentrifuge method.[15] The use of the cuprammonium solvent introduces difficulties, for complexes of unknown structure are formed with the cellulose. Purified cotton linters exhibit molecular weights from 200,000 to 300,000 (*D.P.* from 890 to 1,300). An estimated value for the most mildly treated cellulose leads to a value of 570,000 (*D.P.* 3,500). These weights are all considerably smaller

[14] A. J. Stamm *in* "Wood Chemistry," Editor· L. E. Wise; Reinhold, New York (1944).

[15] H. Staudinger and F. Reinecke, *Papier-Fabr.*, *36*, 489, 557 (1938); H. Staudinger, *ibid.*, *35*, 233 (1937); H. Staudinger and K. Feuerstein, *Ann.*, *526*, 72 (1936).

[16] E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, *39*, 153 (1935).

than those for the larger protein molecules but are appreciably larger than those obtained for cellulose by use of viscosity measurements. Table II gives the results obtained by use of the ultracentrifuge for some typical celluloses and derivatives as reported by Kraemer.[16]

Still higher weights are reported by Gralén and Svedberg[17] for celluloses in cuprammonium solution carefully protected from oxygen. See Table III.

Many measurements have been carried out for cellulose derivatives. A carefully prepared specimen of nitrocellulose was reported by Staudinger

TABLE I

Chain Lengths of Cellulose Preparations as Obtained from Viscosity Measurements in Cuprammonium Solution
(Staudinger[14])

Substance	Average Degree of Polymerization (D P) ($K_m = 5 \times 10^{-4}$)	Molecular Weight (D P × 162)
Cotton (untreated)	2020	330,000
Cotton linters	1440	230,000
Ramie	2660	430,000
Manila	1990	320,000
Bacterial cellulose	1890	310,000
Unbleached spruce cellulose	1360	220,000
Paper pulp—I	730	120,000
Paper pulp—II	870	140,000
Sulfite cellulose		
No. 2503	1360	220,000
No. 2567	405	66,000
Cellophane (Kalle)	280	45,000
Cuprophane (Bemberg)	360	58,000
Viscose rayon	300–400	49,000–65,000
Cuprammonium rayon	400–550	65,000–89,000
Acetate rayon	250–350	

and Schulz[18] to have a molecular weight of about 450,000 (D.P. 2800) as determined by the viscosity method. Commercial nitrocelluloses and cellulose acetates exhibit considerable variation in particle size and are probably much degraded in their preparation. Osmotic pressure measurements indicate that the usual commercial cellulose nitrates and acetates may have degrees of polymerization between the extremes of 25 and 800, although that used in blasting gelatin has, according to Kraemer,[16] a D. P. of about 3000–3500.

[16] E. O. Kraemer, *Ind. Eng. Chem.*, **30**, 1200 (1938).
[17] N. Gralén and The Svedberg, *Nature*, **152**, 625 (1943).
[18] H. Staudinger and G. V. Schulz, *Ber.*, **68**, 2320 (1935).

Separation of a commercial cellulose acetate of the type employed for rayon manufacture was accomplished by fractional precipitation of acetone solutions.[19] The 15 fractions obtained in this manner exhibited degrees of polymerization between the extreme values of 30 and 380 as determined by viscometric measurements.

TABLE II

Molecular Weights of Cellulose and Derivatives from Data Obtained by Use of The Ultracentrifuge

(Kraemer[16])

Material	Molecular Weight	Degree of Polymerization
Native cellulose	>570,000	>3500
Purified cotton linters	150,000 500,000	1000 3000
Wood pulps	90,000-150,000	600-1000
Commercial regenerated cellulose	30,000 90,000	200 600
Fair and Eckerson hydrocellulose	40,000	250
β Celluloses	3 000 15,000	15 90
γ-Celluloses	<3,000	<15
Dynamite nitrocellulose	750,000 875,000	3,000 3,500
Plastics nitrocellulose	125,000 150,000	500 600
½ sec. Nitrocellulose	45 000	175
Commercial cellulose acetates	45 000 100,000	175 360

TABLE III

Molecular Weights of Celluloses

(After Gralén and Svedberg, ultracentrifugal method)

Cellulose	Mol Wt	D P
Unbleached cotton linters	1,500,000	9,200
Raw Georgia cotton	1,000 000	6,200
Nettle fiber cellulose	1,760,000	10,800
Ramie	1,840,000	11,300
Sulfite wood pulp	460,000	2,900

The accepted structure for cellulose (p 532) is seen to contain terminal glucose residues which differ from those in the body of the chain; one end is distinguished by a reducing hemiacetal group and the other by the presence of an extra hydroxyl group at carbon 4. Ideally it should be possible to determine the number of such groups present and thus to estimate the number-average molecular weight of the polymer. Such a method, if accurate,

[19] A. M Sookne, H. A Rutherford, H. Mark and M Harris, *J Research Natl Bur Standards*, **29**, 123 (1942)

would have an advantage over all others since it would not involve the assumptions in regard to the state of solution of the material that must be made when the various physiochemical methods are used.

Unfortunately, the results are far from ideal. In addition to the difficulties encountered in making accurate determinations of groups present in such small concentrations, trouble is caused by any degradative changes which may affect the ends of the chain. For example, very slight oxidative degradation may destroy the terminal aldehyde groups, and stronger oxidation may cleave the chain with the formation of groups of a very different type. On the other hand any hydrolysis which occurs during the estimation of the aldehyde groups will increase the number of such groups and cause the estimates of the molecular weight to be low.

The nonreducing end of the cellulose molecule consists of a glucose unit with four hydroxyl groups. Complete methylation of the cellulose, followed by hydrolysis should result, therefore, in the formation of tetramethylglucose equivalent to the original number of cellulose chains. Not only are difficulties encountered in this method because of change of structure of the end groups through oxidative degradation and by hydrolysis occurring during the course of the analytical procedure, but it is very difficult to determine the small amounts of tetramethylglucose accurately.

The lack of proportionality between viscometric and end group determinations of molecular weight during cellulose degradation has received some consideration.[20] However, it would seem that the results can be at least partially explained without the assumption of complicated structures. First, it has already been pointed out that degradation may cause erroneous results with the end group method. Cellulose degraded by oxidation does not necessarily have the same type of end groups that are present in the original cellulose, and any hydrolysis during the determinations will produce more end groups than were originally present. Second, the quantities measured by the two methods are not the same, and the ratio between them does not necessarily remain constant during degradation. End group determinations measure the number-average molecular weight, which is dependent on different factors from those which influence the weight-average molecular weight as determined by viscometric methods.

As an example of the different results obtained by end group and physiochemical methods, the degrading effect of oxygen during the methylation of cellulose[21] is shown in Table IV.

The degradative effects of small amounts of oxygen remains one of the most puzzling aspects of the accepted structure of cellulose as a chain of

[20] K. Hess and F. Steurer, *Ber*, *73*, 669 (1940); W. N Haworth, *Chemistry & Industry*, (1939) 917; E. Heuser, *Paper Trade J.*, *123*, No 3, 43 (1946).

[21] W. N. Haworth, *Chemistry & Industry*, (1939) 917.

1,4'-glucosidically connected residues, for this type of structure does not suggest any particularly alkali-sensitive groups. Even in trace quantities, oxygen is said to degrade the molecular weight of native cellulose markedly.[22] In its absence, the molecular weights may be as high as 2.5×10^6 (D.P., 15,000). Ultraviolet light, even in the presence of only trace quantities of oxygen, produces molecular degradation, and the degradation reaction shows a post-irradiation effect, i.e., the degradative reaction con-

TABLE IV
Methylation of Cellulose in Nitrogen
(Haworth)

Material	Number and Temp of Methylations	Apparent Chain Length (D P)		
		By End-Group Assay	By Viscosity $t = 0$	By Osmotic Pressure
Cotton slivers	5 at 15°	∞	1300	1300
Cotton linters	3 at 40° and 3 at 55°	∞	600	790
Cotton linters	10 at 15°	∞	340	300
Methylation in Nitrogen and Subsequently in Air				
Cotton slivers	5 in N_2 at 15° 5 in air at 60°	∞	500	400
Cotton linters	3 in N_2 at 40° 3 in air at 60°	∞	240	170
Cotton linters	4 in N_2 at 40° 16 in air at 55°	240	160	120

tinues when the exposure to the ultraviolet light is discontinued.[23] As a result of this treatment, small amounts of water, carbon dioxide and carbon monoxide are liberated.

D. Crystalline Structure.[24] Prior to the development of the chemical structure of cellulose, it had been demonstrated by Nishikawa and Ono that X-ray diagrams could be obtained from natural organic fibers.[25] The dia-

[22] O. P. Golova and V. I. Ivanov, *Bull. acad. sci., U.R.S.S., Classe sci. chim.*, (1945) 279; *Chem. Abst.*, 40, 1653 (1946).

[23] E. Heuser and G. N. Chamberlin, *J. Am. Chem. Soc.*, 68, 79 (1946); R. A. Stillings and R. J. Van Nostrand, *ibid.*, 66, 753 (1944).

[24] H. Mark, *Chem. Revs.*, 26, 169 (1940); W. A. Sisson, *ibid.*, 26, 187 (1940); K. H Meyer, "High Polymers," Vol. 4, p. 236; Interscience Publishers, New York (1942)

[25] S. Nishikawa and S. Ono, *Proc. Phys.-Math. Soc. Japan*, 7, 131 (1913).

grams obtained (see Fig. 1) may vary from a series of concentric circles (which may be sharp or barely distinguishable from a background of continuous radiation) to a more or less sharply symmetrical grouping of spots or arcs. The continuous circles correspond to powder diagrams of simple crystals and indicate merely that the fibers are oriented in the direction of the fiber axis but have random orientation in directions perpendicular to this axis. The arcs are given by materials more highly orientated in directions perpendicular to the fiber axis and spots are obtained when the molecules lie parallel in both directions. Certain natural sources of cellulose

(A)   (B)
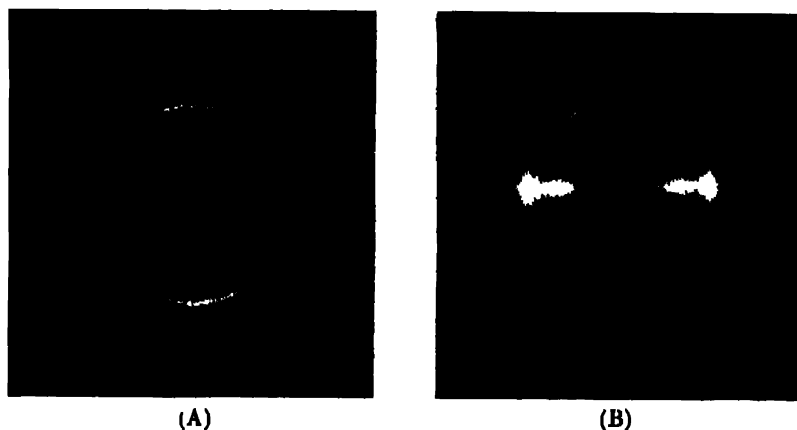
Fig. 1. X-Ray diffraction patterns of cotton cellulose (A) and ramie cellulose (B). (Furnished by courtesy of Milton Harris.)

e.g., the cell walls of the alga *Valonia ventricosa*, contain the substance in a highly oriented condition. Orientation also may be induced by mechanical means such as stretching films or rolling fibers.

a. Unit Cell

Early attempts at the evaluation of the X-ray diffraction data led to an elementary cell of the rhombic type, but later a monoclinic cell was proposed by Meyer and Mark. A monoclinic crystal has three unequal axes, one of which (b-axis) is at right angles to the plane of the other two axes (*a, c*). The angle between the *a* and *c* axes is indicated as β and cannot be a right angle. A painstaking investigation of the problem by Gross and Clark[28] confirms the allocation of the unit cell to the monoclinic system and provides the following values for the dimensions of the cell:

$$a = 8.35\ \mathring{A},\ b = 10.3\ \mathring{A},\ c = 7.95\ \mathring{A},\ \beta = 84°.$$

[28] S. T. Gross and G. L. Clark, *Z. Krist.*, **99**, 357 (1938).

Of the various monoclinic crystal classes and space groups of these classes, the data best fits C_2^3. From the calculated volume of the unit cell and the known density of cellulose, it is calculated that four glucose residues are contained in this volume.

b. SPATIAL MOLECULAR MODEL

The intensity of the spots of the diffraction pattern cannot be utilized directly for locating the spatial positions of the various atoms of the molecule, for too many atoms are present and the scattering power of oxygen and carbon atoms is about the same. Instead, spatial models are prepared until one is found which agrees with the observed intensities of the X-ray diffraction pattern and fits into the unit cell. Sponsler and Dore[27] suggested the first model of this type, based on a chain polymeric structure for cellulose, but their model was derived from the incorrect assumption of 1-1' and 1'-4" bonds between the glucose residues. Later this model was modified by utilizing 1,4' disaccharide bridges between the various glucose residues.[28] Originally, it was assumed by Meyer and Mark that all of the chains have the same orientation with respect to the fiber axis; later it was found necessary to place half of the chains running in one direction and half in the other direction but in both instances parallel to the fiber axis. The accompanying figures (2 to 5), first published by Meyer and Misch[29] present the structure of the unit cell of cellulose as obtained from X-ray and chemical data.

Along the fiber axis (b-axis) the identity period is 10.3 Å. As this figure represents the size of one fully extended cellobiose unit, the molecular chains must run parallel to the fiber axis. (See Fig. 2). One chain runs along the b-axis (fiber axis) and is surrounded by four chains at the corners of the unit cell; the chains run parallel but have the opposite sequence of "tail groups" and ring oxygen atoms.

Each unit cell contains four glucose residues. These residues comprise the two along the b axis and $\frac{1}{8}$ of each of the eight glucose residues located on the corners of the unit cell. Those at the corners are shared by each of the four unit cells having common corners. In this direction (along the fiber axis), the chains are held together by carbon-oxygen glucosidic bonds with energies of the order of 50,000 calories per mole.

According to the model, in the direction of the c-axis, the distance between carbon atoms and oxygen atoms of neighboring chains is about 3.1 Å. In the same direction the hydroxyls are situated about 3.8 Å from one another. These distances approximate those which would be expected if the

[27] O. L. Sponsler and W. H Dore, *Colloid Symposium Monograph, 4*, 174 (1926).

[28] K. H. Meyer and H Mark, *Ber., 61*, 593 (1928); *Z. physik. Chem., (B) 2*, 115 (1929).

[29] K. H Meyer and L. Misch, *Helv. Chim. Acta, 20*, 232 (1937). For later suggestions of improvements, see F. T. Pierce, *Nature, 153*, 586 (1944); P H. Hermans, *Kolloid Z , 102*, 169 (1943).

stabilizing force in this direction was of the van der Waals' type. Hence, it is probable that in the direction of the c-axis the chains of the crystallites are stabilized by van der Waals' forces which amount to about 8000 cal. per mole.

Along the a-axis, the oxygen atoms of neighboring chains approach each other much closer (2.5 $\overset{\circ}{A}$) than is possible if purely van der Waals' forces are responsible (3.0 $\overset{\circ}{A}$). It seems probable that in this direction, the crystals are stabilized by hydrogen bonds with energy of the order of 15,000 cal. per
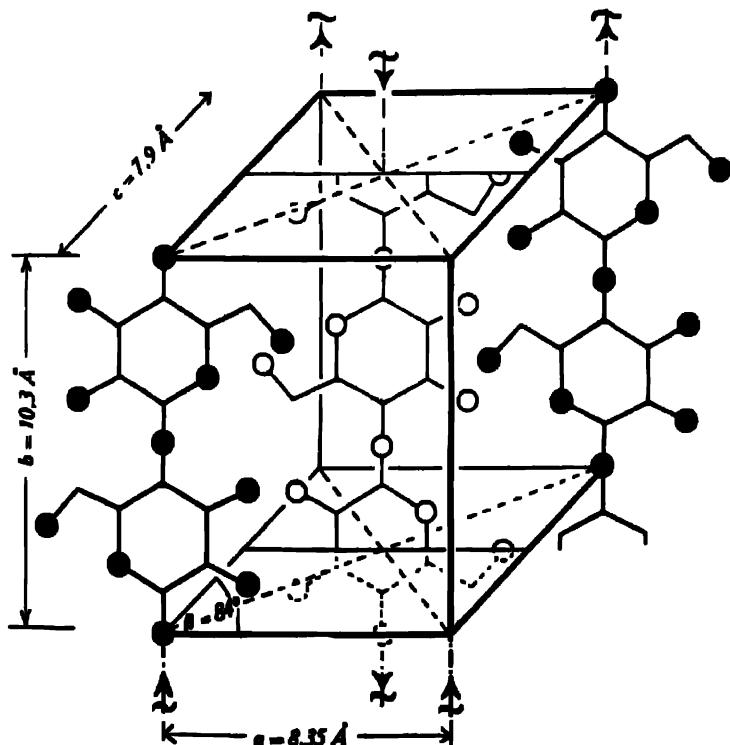
FIG. 2. Spatial representation of the unit cell of cellulose.   (After Meyer and Misch.)

mole. Infrared absorption studies of cellulose show that relatively few "unperturbed" hydroxyl groups are present.[30]

c. MICELLAR STRUCTURE OF NATIVE CELLULOSE FIBERS

The unit cells described above are built up in the cellulose fiber to form crystalline areas called "micelles" or "crystallites," interspersed with non-crystalline (amorphous) areas. The extent of orientation has a profound effect on the reactivity of undissolved fibers, and on the mechanical and

[30] J. W Ellis and J Bath, *J. Am Chem. Soc*, *62*, 2859 (1940)

aging properties of derived plastic materials.[31] The breadths of the lines of cellulose X-ray diffraction diagrams have been utilized for estimating the crystallinity.[32] Ordinarily, the method leads only to minimal values, and in any case many assumptions must be made. The intensity of the central spot of diffraction diagrams also may be employed for this purpose.[33] Other methods used for the determination of the relative degree of crystallinity of cellulose are based on the integral heats of wetting of the material and of the sorptive capacity for water at known relative humidities.[34]

Fig. 3. Projection of the unit cell of cellulose along the c axis and ab plane perpendicular to c. (After Meyer and Misch.)

The reaction of thallous ethylate with cellulose in non-swelling liquids (ether or benzene) appears to allow combination only with hydroxyl groups in amorphous areas. Subsequent treatment with methyl iodide give a product in which the number of methyl groups will be a measure of the degree of reaction with the thallous ethylate.[35]

[31] W. O. Baker, *Ind. Eng. Chem.*, *37*, 246 (1945).

[32] J. Heugstenberg and H. Mark, *Z. Krist.*, *69*, 271 (1929); M. von Laue, *ibid.*, *64*, 115 (1926).

[33] R. Hosemann, *Z. Physik*, *113*, 751 (1939); *114*, 133 (1939).

[34] See: P. H. Hermans and A. Weidinger, *J. Am. Chem. Soc.*, *68*, 1138 (1946); A. G. Assaf, R. H. Haas and C. B. Purves, *ibid.*, *66*, 66 (1944).

[35] A. G. Assaf, R. H. Haas and C. B. Purves, *J. Am. Chem. Soc.*, *66*, 59 (1944).
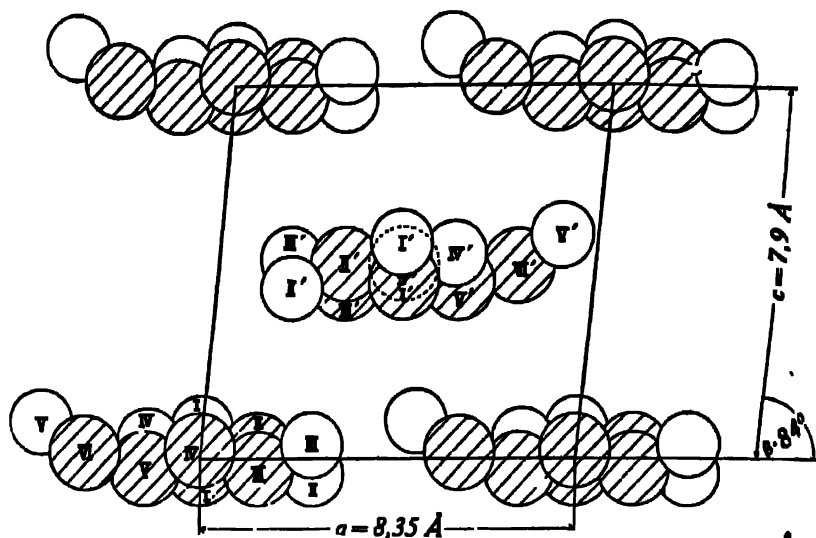
FIG. 4. Projection of the unit cell along the b axis and on the ac plane perpendicular to b. (After Meyer and Misch.)
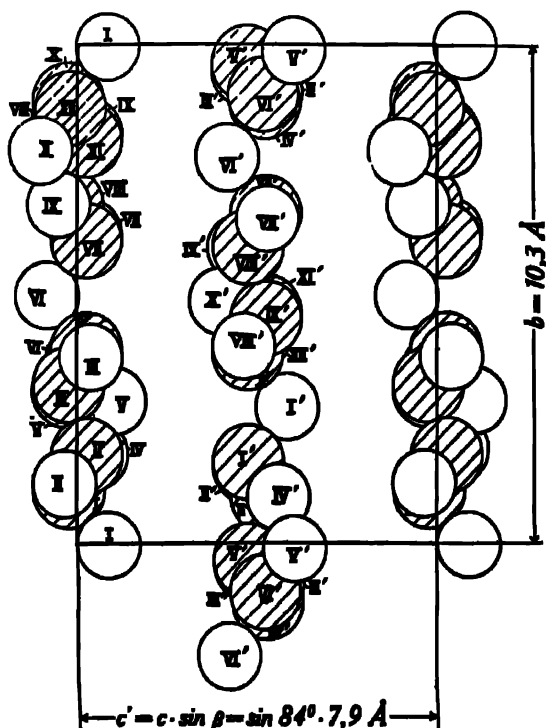
FIG. 5. Projection of the unit cell along the a axis and on the bc plane perpendicular to a. (After Meyer and Misch.)

$$\overset{|}{HC}{-}OH + TlOEt \longrightarrow \overset{|}{HC}{-}OTl \xrightarrow{\ CH_3I\ } \overset{|}{HC}{-}OCH_3 + TlI$$
$$+$$
$$EtOH$$
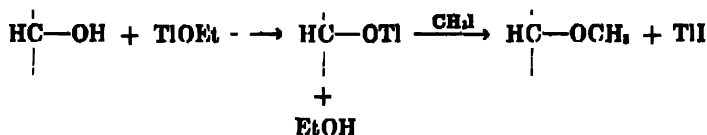
By this method, it was demonstrated that the amorphous fraction of swollen cotton linters is as high as 27% whereas the amount in unswollen fibers is about 0.25 to 0.5%.

d. Regenerated Cellulose

When cellulose is precipitated from solution, its crystalline form, as shown by X-ray diffraction studies, differs from that of native cellulose. This allotropic modification, termed regenerated or hydrated cellulose, is the form in which most artificial fibers (rayon), artificial membranes (cellophane) and mercerized cotton exist. The dimensions of the unit cell,[36] which belong to the monoclinic system, have been determined as:

$$a = 8.1\ \text{Å}, b = 10.3\ \text{Å}, c = 9.1\ \text{Å}, \beta = 62°.$$

Regenerated cellulose may be converted to native cellulose by treatment with water or glycerol at 140 to 300°C. When alkali cellulose is decomposed by hot water, native cellulose is produced; but with cold water, the regenerated form is obtained.[37] It seems probable that the stable modification is the native form, but at the lower temperatures the velocity of interconversion is too small to be observable.

2. Reactions of Cellulose[38]

Two general types of reactions of cellulose have been recognized. In the first individual crystallites are not destroyed, and the reaction takes place at the surface of the micelles (crystallites). These reactions are principally absorptive processes, e.g., the sorption of water, metallic salts and dyes and may be accompanied by swelling. In the second type, the fiber structure is broken down into the component chains before reaction takes place. An important intermediate type of reaction involves a surface chemical reaction accompanied by a partial penetration of the crystal lattice. The nature of a particular reaction usually may be decided from a study of the X-ray pattern of the initial and final products. Absorptive processes, in most in-

[36] K. R. Andress, Z. physik. Chem., (B) 4, 190 (1929). See also: O. L. Sponsler and W. H. Dore, J. Am. Chem. Soc., 50, 1940 (1928); E. Sauter, Z. physik. Chem., 37, 161 (1937).

[37] K. H. Meyer, L. Misch and N. P. Badenhuizen, Helv. Chim. Acta, 22, 59 (1939).

[38] H. M. Spurlin, "Cellulose and Cellulose Derivatives," (E. Ott, Editor) p. 607; Interscience Publishers, New York (1943). E. C. Jahn, "Wood Chemistry," (L. E. Wise, Editor), p. 762; Reinhold, New York (1944).

stances, do not affect the diffraction pattern, but a penetration and change of the crystallite lattice structure is readily recognizable by a change in the pattern.

From the standpoint of its reactions, cellulose differs from the simple sugars and oligosaccharides in its limited solubility and in the almost complete absence of unsubstituted hemiacetal groups. The principal chemical reactions of cellulose involve the substitution of one or more of the three hydroxyl groups of each glucose residue and the hydrolysis of the glucosidic linkages between the glucose residues. Unless substitution reactions take place under carefully controlled and mild conditions, both kinds of reaction may occur simultaneously with resulting degradation of the molecular size.

A. Reactions with Bases, Acids and Salts. These reactions are of great industrial importance, for they provide means for bringing cellulose into solution and then regenerating it as fibers, sheets, etc.

a. AMMONIA, AMINES, QUATERNARY BASES, ETC.

Cellulose absorbs gaseous ammonia without distortion of the diffraction pattern.[39] Liquid ammonia, however, forms a crystalline compound with the polysaccharide. The lattice is penetrated, for the ammonia-cellulose exhibits a new diffraction pattern.[40] In an aqueous solution of hydrazine or ethylenediamine, cellulose swells; at high amine concentrations, some penetration of the lattice takes place. Tetraethylammonium hydroxide, benzyltrimethylammonium hydroxide and some other quaternary bases dissolve cellulose, but each of these organic bases exerts its maximum solubilizing effect at a definite concentration (about 2 N) which decreases with the molecular weight of the base.[41]

The solubility of cellulose in ammoniacal solutions of copper oxide (Schweizer's reagent) is well known, and extensive use has been made of this property in the manufacture of synthetic fibers (cuprammonium rayon) and in the determination of molecular weights by the methods previously described. The cuprammonium reagent produces very little degradation of the cellulose, and in the absence of oxygen and light, the regenerated cellulose obtained by the addition of acids or alkalies to the solution is only slightly degraded.[42]

Copper oxide dissolves in the ammonia solution due to the formation of cuprammonium hydroxide, $Cu(NH_3)_4(OH)_2$. Dissolution of cellulose in the solution takes place with the liberation of ammonia from the complex ion, and presumably the cellulose takes the place of the ammonia in the

[39] N. H. Grace and O. Maass, *J. Phys. Chem.*, **96**, 3046 (1932).
[40] A. J. Barry, F. C. Peterson and A. J. King, *J. Am. Chem. Soc.*, **58**, 333 (1936); K. Hess and J. Gundermann, *Ber.*, **70**, 1788 (1937).
[41] T. Lieser, *Ann.*, **528**, 276 (1937).
[42] H. Staudinger and B. Ritzenthaler, *Ber.*, **68**, 1225 (1935).

complex ion.[43] Similar compounds are formed by cuprammonium solution and simple polyalcohols, and Fehling solution probably has an analogous composition.

The case of oxidation of cuprammonium solutions of cellulose prevents their use for some purposes. In such instances, aqueous solutions of cupric hydroxide-ethylenediamine provide excellent solvent media because of the stability of the cellulose in this solvent.[44]

The action of sodium hydroxide has particular importance because it is involved in the preparation of mercerized cotton, viscose and certain cellulose ethers. At concentrations of the sodium hydroxide less than 8 to 9%, the reaction apparently takes place at the surface of the micelles since the X-ray diagram is not affected. The combined alkali at this stage averages one mole for each two glucose residues. The X-ray diagrams of cellulose treated at higher concentrations of sodium hydroxide exhibit a new diagram superimposed upon that for the original cellulose, and, at alkali concentrations in the range 13 to 19% , only the new diagram is obtained. At this stage, the combined alkali averages one mole per glucose residue. Still another diagram is obtained when the concentration of sodium hydroxide is raised to 21%. The nature of the combination is uncertain; at the lower concentrations adsorption probably occurs, whereas at the higher concentrations the formation of alcoholates and cellulose anions may take place.

$$\begin{matrix} & \text{H} \\ & | \\ \text{R} & \text{O} \quad \text{Na}^+, \quad \text{R} \quad \text{O} \quad \text{Na (alcoholate)}, \quad \text{R} \ -\text{O}^- \ \text{Na}^+ \ \text{(cellulose anion)} \end{matrix}$$

The reaction is markedly affected by the temperature, and the same effect is produced by a 6.5% solution at $-10°$ as by a 17 to 18% solution at 20°.

Cold water decomposes the alkali cellulose with the formation of hydrate cellulose. Hot water gives a mixture of native and hydrate cellulose.

The preparation of mercerized cotton involves treatment of cotton fibers with strong sodium hydroxide while the fibers are kept under tension to prevent shrinking. The mercerized cellulose obtained by treatment of the alkali cellulose with water has a smooth, lustrous appearance and takes up dyes better than the untreated material.

In the glucose units of the cellulose chain, the hydroxyls of carbons 2 are the most acidic. Thus, in 35% potassium hydroxide solutions, a compound with the formula $C_6H_{10}O_5 \cdot KOH$ is formed. The potassium seems to be associated with carbon 2 since methylation of the compound and hy-

[43] For discussion of structure of complex see: T. Lieser and H. Swiatkowski, *Ann.*, **538**, 110 (1939); L. J. Jolley, *J. Textile Inst.*, **30**, T4 (1939).

[44] F. L. Straus and R. M. Levy, *Paper Trade J.*, *114*, No. 3, 31, No. 18, 33 (1942); R. S. Hatch, *Ind. Eng. Chem.*, *Anal. Ed.*, *16*, 104 (1944).

drolysis yield 2-methylglucose.[46] Similar experiments carried out with the sodium cupricellulose compound gave 2-methyl- and 3-methylglucose. All the free hydroxyls of cellulose are weakly ionizable as is shown by the complete exchange which takes place in deuterium oxide.[46] The exchange is virtually complete in 30 hours at 30°C.

Although the nature of the combination between sodium hydroxide and cellulose in the alkali (soda) cellulose remains undecided, true trisodium alcoholate derivatives have been described. Their preparation involves the reaction of cellulose with a solution of metallic sodium in liquid ammonia.[47] When exposed to air and moisture, these derivatives undergo decomposition and degradation of the molecular chains.

Hydroxides of the other alkali metals (Li, K, Cs, Rb) form alkali celluloses similar to those obtained by the use of sodium hydroxide.[48] The maximum swelling effect is produced at a definite concentration for each base and increases with the atomic weight of the alkali metal involved. The product obtained by use of lithium and potassium hydroxides has a composition averaging that of one mole of alkali for each pair of glucose residues, but that from the action of cesium and rubidium hydroxides averages one mole of base to three glucose residues.

b. Action of Inorganic Acids and Salts

At low acid concentrations, cellulose swells, but the X-ray diagram remains unchanged. However, a change is observed for concentrations of hydrochloric acid greater than 24%, and at a concentration of 41% the cellulose dissolves rapidly. Sulfuric acid (10.5 moles per liter) and phosphoric acid (14.1 moles per liter) also act as solvents. Thiocyanates, lithium halides, zinc chloride and other salts in dilute solution produce swelling of cellulose and dissolution at higher concentrations. Since the cellulose molecules probably are held in the micellar lattice by hydrogen bonds and van der Waals' forces, the solubilizing action of strong acids and salts probably is due to a replacement of bonds such as —C—O— H- O-

$$
\begin{array}{c}
\text{H} \\
|
\end{array}
$$

by —C—O--Li$^+$. At proper concentrations, crystalline addition products are formed from cellulose and substances such as nitric acid, perchloric acid[49] and lithium thiocyanate. These addition compounds exhibit charac-

[46] W. J. Heddle and E. G. V. Percival, *J. Chem. Soc.*, 1690 (1938); 249 (1930).

[46] G. Champetier and R. Viallard, *Bull. soc. chim.*, [5] 5, 1042 (1938).

[47] P. C. Scherer, Jr., and R. E. Hussey, *J. Am. Chem. Soc.*, 53, 2344 (1931); P. Schorigin and N. N. Makarowa-Semljanskaja, *Ber.*, 69, 1713 (1936).

[48] E. Heuser and R. Bartunek, *Cellulosechem.*, 6, 19 (1925); T. Lieser, L. Henrich and F. Fichtner, *Ann.*, 538, 99 (1939).

[49] See T. Lieser and F. Fichtner, *Ann.*, 548, 195 (1941).

teristic X-ray diagrams. From solutions of cellulose in acids, regenerated cellulose is obtained by dilution with cold water. Since the product gives a blue color with iodine in the presence of traces of sulfuric acid, it is sometimes called "amyloid."

The action of acids and salts is accompanied by a degradation of the chains due to hydrolysis of the glucosidic linkages. The decrease in viscosity of solutions of the regenerated product and the increase in reducing power are proof that hydrolysis has taken place. Phosphoric acid (86 per cent) produces considerably less hydrolysis than sulfuric acid or concentrated hydrochloric acid.[50] The degradation by hydrochloric and sulfuric acid takes place by a progressive breakdown of the molecule. Substances of intermediate chain length (cellodextrins) are the primary hydrolysis products, but finally oligosaccharides and glucose are obtained. Crystalline cellohexaose, cellotetraose, cellotriose and cellobiose have been isolated from the products of hydrolysis.[51] Mild hydrolytic conditions lead to the formation of "hydrocellulose," which has a diminished chain length as is demonstrated by its low viscosity. It is sometimes defined as the material which dissolves in 8% sodium hydroxide. The decrease in chain length is also reflected in the practically complete loss of fiber strength. After additional hydrolysis, water-soluble "cellodextrins" of high reducing power are produced. These products probably are not homogeneous substances.

B. Cellulose Xanthate. Under the influence of carbon disulfide and barium hydroxide, methyl α-glucoside reacts to form the monoxanthate. The xanthate is a mono ester of dithiocarbonic acid:

$$R—O—\overset{\shortmid}{C}—S—Na$$
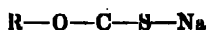
Small yields of the di- and tri-xanthates are also obtained. The yields of the di- and tri-esters can be increased by using phenyl β-glucoside and, as the base, tetramethylammonium hydroxide.[52]

Cellulose forms similar xanthates which are of considerable industrial importance for the preparation of synthetic fibers by the viscose process. Cellulose pulp is first treated with 17 to 18% sodium hydroxide solution. The alkali cellulose is freed from the solution and allowed to stand ("age") in contact with air. The "aging" process degrades the chains and must be interrupted at the proper time since too mild treatment produces products of high viscosity and too much degradation results subsequently in the production of fibers with low strength. The properly "aged" alkali

[50] A. J. Stamm and W. E. Cohen, *J. Phys. Chem.*, *42*, 921 (1938); A. Ekenstam, *Ber.*, *69*, 549, 553 (1936).

[51] R. Willstätter and L. Zechmeister, *Ber.*, *62*, 722 (1929); L. Zechmeister and G. Tóth, *ibid.*, *64*, 854 (1931).

[52] T. Lieser and Associates; see: *Ann.*, *528*, 48 (1936); *Papier-Fabr.*, *36*, 272 (1938).

cellulose upon exposure to carbon disulfide vapor is converted to a yellow-orange, viscous mass (viscose) of cellulose xanthate. When the viscose is allowed to stand or "ripen," an initial period of decreasing viscosity is followed by a period during which the viscosity increases; these changes represent changes in the structure of the gel, for the molecular weight remains essentially constant. The presence of air speeds up this "ripening" process. Forcing the "ripened" viscose through small openings into an acid bath regenerates the cellulose with the liberation of carbon disulfide. By the use of fine orifices, threads are obtained whereas fine slots produce thin sheets of regenerated cellulose (cellophane).

The composition of the cellulose xanthate corresponds to that of one xanthate group for each two glucose residues. Since the X-ray diagram remains unchanged from that for alkali cellulose, a real compound probably is not formed, for the micellar lattice is not penetrated. The replacement of the sodium hydroxide by tetraethylammonium hydroxide leads to a cellulose trixanthate. The xanthate group of the usual compound has been shown[12] by methylation studies to be associated with carbon 2. Diazomethane replaces the xanthate by a methyl group, and hydrolysis of this methylated cellulose produces glucose and 2-methylglucose.

C. Oxycellulose. Celluloses, particularly under alkaline conditions, are readily oxidized even by such mild agents as atmospheric oxygen. The oxidized celluloses (oxycelluloses) are of considerable industrial interest, but the nature of the oxidations, in most cases, is not well understood. It may take place by oxidation of terminal hemiacetal groups ("aldehyde" groups), of secondary glycol units (at carbons 2 and 3 of each glucose unit) and of the terminal primary hydroxyl groups.

Oxidation of the hemiacetal groups results from the action of iodine in alkaline solution and from the action of chlorous acid. Iodine in alkaline solution has been used for the estimation of aldehyde groups in hydrocelluloses and oxycelluloses. Except for a small correction, the reaction is said to take place stoichiometrically,[13] but most workers have found that secondary oxidations take place.

Under alkaline conditions, atmospheric oxygen reacts with cellulose probably by simultaneous oxidation of the hemiacetal groups and of the products of the interconversion induced by the alkali (see discussion of the action of alkalies on sugars, p. 71). This type of reaction is thought to be responsible for the "aging" of viscose (p. 546). The degradations which occur when cellulose is methylated in air, or when it is dissolved in cuprammonium solutions and oxygen is not excluded, probably are of a similar character.

Periodic acid is one of the most specific oxidative reagents for cellu-

[12] See: H. A. Rutherford, F. W. Minor, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **29**, 131 (1942).

lose.[53,54] The reagent attacks the glycol groupings at carbons 2 and 3, cleaves the carbon bond and forms aldehyde groups (see p. 328). However, considerably more than the theoretical amount of oxygen is consumed, and some formaldehyde, formic acid and carbon dioxide are produced. The secondary reaction may arise from the action of alkali on the dialdehyde units, the presence of which makes the oxycelluloses extremely sensitive to alkaline hydrolysis.

Nitrogen dioxide (NO_2) brings about a fairly specific oxidation of primary alcoholic groups in cellulose to carboxyl groups[55] (see Chapter VII). Oxycelluloses of this type (polyglucuronic acids) are commercial products; they are analogous in structure to pectins and are readily degraded by alkalies.

Other common oxidation reagents appear to act much less selectively, and it is probable that the three above-mentioned types of oxidation occur simultaneously with perhaps still other types such as the oxidation of secondary alcoholic groups to keto groups. The properties of the oxycellulose will depend upon the major type that has occurred. Glycol cleavage produces products with high reducing power and sensitivity to alkaline hydrolysis. Products containing carboxyl groups fix basic dyes and show some sensitivity to alkaline conditions.[55]

A number of methods have been devised for the determination of the nature of the groups formed in the oxidation of cellulose.[57] These include:

1. Determination of carbonyl groups by titration with hydroxylamine and of aldehyde groups by oxidation with hypoiodite or by reaction with diamines and subsequent fixation of dyes.

2. Determination of uronic acid residues by evolution of carbon dioxide.

3. Measurement of residual hydroxyl groups by acetylation or nitration.

4. Measurement of carboxyl groups by direct titration or by partition methods based on calcium acetate, silver phenolates or methylene blue.

D. Cellulose Esters.[58] The esters formed by the esterification of the hydroxyl groups in cellulose are of great industrial importance and find many uses, especially as plastics, textile fibers, explosives, transparent films and lacquers. Nitrocellulose is the only inorganic ester which is important commercially, and cellulose acetate has found much wider applica

[53] E. L. Jackson and C. S. Hudson, *J. Am. Chem. Soc.*, **60**, 989 (1938); G. F. Davidson, *Shirley Inst. Memoirs*, **18**, 69 (1941).

[54] E. C. Yackel and W. O. Kenyon, *J. Am. Chem. Soc.*, **64**, 121 (1942); C. C. Unruh and W. O. Kenyon, *ibid.*, **64**, 127 (1942).

[55] See, for example, the results for chromic acid oxidation: G. F. Davidson, *Shirley Inst. Memoirs*, **18**, 91 (1941).

[57] C. C. Unruh and W. O. Kenyon, *Textile Research J.*, **16**, 1 (1946); E. Geiger and A. Wissler, *Helv. Chim. Acta*, **28**, 1639 (1945); E. Geiger, *ibid*, **28**, 1159, 283 (1945); F. Müller, *ibid.*, **29**, 130 (1946).

[58] C. R. Fordyce, *Advances in Carbohydrate Chem.*, **1**, 309 (1945).

tion than any of the other organic esters. Although there is some debate as to whether the nitration reaction is heterogeneous or homogeneous (with the nitrating mixture penetrating uniformly to all parts of the fiber), acylation of cellulose is known to be heterogeneous. The cellulose does not go into solution until esterification is practically complete and the crystalloids are broken down. Esters with less than the maximum degree of substitution must be prepared by partial hydrolysis of the trisubstituted product if they are to be completely soluble and the ester groups are to be distributed over the full length of the chain.[59] For example, commercial acetone-soluble cellulose acetates which average 2.1 to 2.6 acetyl groups per glucose unit are prepared by partial hydrolysis of cellulose triacetate. Such hydrolyses are among the few cases in which cellulose or one of its derivatives undergoes reaction in a perfectly homogeneous solution. Other cases include the etherification of cellulose dissolved in aqueous alkali or cuprammonium solution and the substitution of the free hydroxyls in secondary acetates by higher fatty acids or dibasic acid radicals.

With simple alcohols of low molecular weight, the primary alcohols are esterified about ten times as fast as the secondary alcohols.[60] Although such a difference in reactivity between the two types of hydroxyl groups may carry over to the acetylation of cellulose, it is undoubtedly much less important than the position of the group in the micelle. The relative reactivity of the free hydroxyl groups in soluble, partially acetylated products is not complicated by micelle structure, but it may be considerably influenced by the steric effects of the acetyl groups present. The relative rates of tosylation of a soluble cellulose acetate with a degree of substitution of 2.44 were found to be 2.2, 0.11, and 23 for the hydroxyls at positions 2, 3, and 6, respectively.[61] This difference in reactivity between the various positions was probably markedly influenced by the acetyl groups already present and also by the size of entering tosyl groups, especially in the case of the hydroxyl at carbon 3, where the low reactivity may well be caused by the steric effect of a substituent on carbon 2.

a. CELLULOSE NITRATES

By the action of fuming nitric acid, or mixtures of nitric acid and a catalyst such as concentrated sulfuric acid, nitric acid esters of cellulose (incorrectly called nitrocelluloses) are obtained. The theoretical nitrogen content of 14.17% for a completely esterified product (cellulose trinitrate) seldom is attained. The commercial products termed pyroxylin contain

[59] See for example, M. E. Martin and T. M. Andrews, U. S. Patent 2,373,630, April 10, 1915.

[60] H. M. Spurlin, *J. Am. Chem. Soc.*, **61**, 2222 (1939).

[61] T. S. Gardner and C. B. Purves, *J. Am. Chem. Soc.*, **64**, 1539 (1942).

10.5 to 12.5% nitrogen, but smokeless powder is more highly nitrated (12.5 to 13.5%). The use of phosphoric acid as a catalyst is claimed to lead to a cellulose trinitrate with the theoretical nitrogen content.[62] The reaction may be represented by the equation:

$$\text{R--C- OH} + \text{HONO}_2 + (\text{H}_2\text{SO}_4) \rightleftharpoons \text{R--C---ONO}_2 + (\text{H}_2\text{SO}_4) + \text{H}_2\text{O}$$
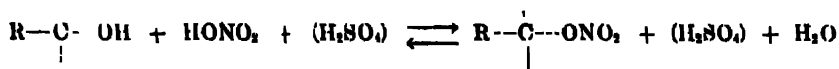
As is evident from the formation of water as one of the products of the esterification process, the water concentration is one of the most important factors in determining the position of the final equilibrium as represented by the degree of nitration. The temperature must be kept below 40°C., or appreciable degradation of the cellulose chain results. In the preparation of nitrocellulose (pyroxylin) for use in plastics and lacquers, wood cellulose is chosen because of its lower cost. Technical improvements in the purification process make it possible to use wood cellulose for the preparation of explosives, whereas previously cotton had been the only raw material considered for this purpose.

Up to a nitrogen content of about 7.5%, the fibers have the lattice of hydrated cellulose. The X-ray diagram becomes diffuse at increasing contents of nitrogen, and above 12.7% nitrogen, the diagram of cellulose trinitrate appears.[63]

The nitration reaction is complicated by several secondary reactions. In addition to some hydrolysis of glucosidic linkages, a few of the hydroxyl groups are esterified by sulfuric acid with the formation of mixed nitrates and sulfates. The formation of these mixed esters may provide an explanation for the difficulty of attaining complete nitration. These sulfate esters also may be intermediates in the reaction. The sulfate groups, however, are easily hydrolyzed by boiling with water. The removal of these groups probably explains the "stabilization" of nitrocelluloses which is brought about by treatment with very dilute acids and boiling water. Another possible intermediate in the nitrating reaction is Knecht's compound, named after its discoverer. This compound, which has a different lattice from that of cellulose, is formed in nitric acid as dilute as 62%. The formula[64] has been variously described as: $C_6H_{10}O_5\cdot HNO_3$, $C_6H_{10}O_5\cdot HNO_3\cdot H_2O$, and $(C_6H_{10}O_5)_2\cdot HNO_3$.

Pyroxylin (10.5 to 12.5% nitrogen) has important industrial application

[62] E. Berl and G. Rueff, Cellulosechem., 12, 53 (1931); see, however, T. Tomonari, ibid., 17, 29 (1936); E. Berl, U. S. Patent 2,384,415, Sept. 4, 1945.

[63] F. D. Miles et al., J. Phys. Chem., 34, 2607 (1934); M. Mathieu, Compt. rend., 200, 143 (1935).

[64] C. Trogus, Cellulosechem., 15, 104 (1934); G. Champetier and R. Marton, Bull. soc. chim., [5] 10, 102 (1943); Chem. Abst., 38, 2483 (1944).

in the preparations of plastics (celluloid), lacquers, films and artificial leather. The chain lengths of the nitrocelluloses used in these applications are considerably shorter than those of natural fibers, but this degradation is a necessary condition to obtain products with the proper viscosity. Inasmuch as the presence of small amounts of the long chains greatly increases the viscosity whereas short chains have an undesirable influence on the mechanical strength, the products should be fairly homogeneous. By heating nitro celluloses with water under pressure, products of low viscosity are obtained.

Celluloid is prepared by mixing moist pyroxylin, camphor, alcohol and pigments. By the application of heat and pressure, the resulting mass is forced into molds and shaped. Rather a tight complex is formed between the nitrocellulose and the camphor. Since the nitrocellulose which forms the best bond with the camphor has one free hydroxyl per glucose group and since the composition of complete complex formation is one camphor molecule per glucose unit, it seems probable that the complex is formed by hydrogen bonding between the two groups.[66] Camphor eliminates the explosive properties, but leaves the product very inflammable. Celluloid for use in photographic films has a much lower camphor content. For the preparation of celluloid films, a thick solution of pyroxylin and camphor in methanol, ethyl acetate and other solvents is allowed to flow on to the circumference of a large wheel. The solvent evaporates during a single revolution of the wheel and a continuous film may be removed.

Lacquers for automobiles and interior decoration are composed of low viscosity pyroxylin, a solvent such as the esters of acetic acid and a plasticizer (e. g., castor oil, camphor, tricresyl phosphate, etc.). Without the plasticizer, the film formed after evaporation of the solvent wrinkles and buckles away from the surface. Artificial leathers are made by coating cotton fabrics with lacquer and then embossing the material to simulate leather.

Although the ester linkages may be hydrolyzed by the action of strong sulfuric acid, considerable degradation takes place. The use of alkali leads to oxidative decomposition, but alkali sulfides may be successfully employed to hydrolyze the nitrate groups (p. 177). The use of alkali sulfides was of considerable importance at one time in the Chardonnet process for artificial silk. Pyroxylin in a solution of equal volumes of ether and alcohol (collodion) was forced through fine openings and the solvents were removed in a current of air. The thread formed by combining a number of fine filaments was subjected to the action of sodium acid sulfide (NaHS) which removed the nitrate groups and regenerated the cellulose. The method has considerable historical interest since it is one of the first, if not the original method, for making rayons.

[66] M. Wadano, K. Hess and C. Trogus, Z. physik. Chem , 30, 159, 183, 232 (1935).

b. Cellulose Acetates

The free hydroxyls of cellulose may be acetylated in a manner similar to that for the simple sugars (p. 150). The cellulose is treated with acetic acid, acetic anhydride and a catalyst. As a catalyst, sulfur dioxide, sulfuric acid or occasionally zinc chloride is employed. Perchloric acid has been shown to have certain advantages for this purpose, but its corrosiveness limits its application.[66] Complete acetylation with the formation of a product containing 62.5% combined acetic acid (44.8% acetyl) is more easily attained than is complete nitration. On the other hand, a greater degradation of the cellulose chains takes place in acetylation than in nitration. However, by the action of pyridine and acetic anhydride for long periods of time at high temperatures, it is possible to obtain acetates without a simultaneous shortening of the chains.[67]

The completely acetylated product (triester) is soluble in glacial acetic acid, chloroform, dichloroethane and a few other solvents. By partial hydrolysis involving the removal of about one-sixth of the acetate groups, "secondary" acetates soluble in acetone are obtained. Complete saponification by acids or alkalies gives regenerated cellulose in a more or less degraded condition.

For commercial operations, sulfuric acid is the most common catalyst. In the intermediate stages of acetylation, the sulfuric acid combines quantitatively with the cellulose in the form of the acid sulfate.[68] In the final stages, the acid sulfate groups are gradually replaced by acetyl groups, but the final product contains some combined sulfur. During acid hydrolysis of the fully acetylated material, the combined sulfur drops to about 0.01%.

Cellulose acetates have considerable industrial importance, e.g., as acetate rayon and as plastics, lacquers and films including photographic film of narrow width for which curling is not serious. Their principal advantages over the nitrates arises from their nonflammability, transparency, light resistance and suitability for injection molding in plastic mixtures. However, the higher viscosity and cost retard their general replacement of cellulose nitrates for these purposes. Acetate silk is made by dissolving "secondary" acetate, with an acetic acid content of about 52 to 56%, in acetone and forcing the solution through spinnerets into a stream of warm air. The solvent evaporates leaving solid threads. For the preparation of plastics, the process is similar to that for cellulose nitrate; but tricresyl phosphate, triphenyl phosphate, dibutyl phthalate and other esters are used

[66] D. Krueger and E. Tschirch, Ber., 64, 1874 (1931).

[67] K. Hess and N. Ljubitsch, Ber., 61, 1460 (1928); H. Staudinger and G. Daumiller, Ann., 529, 219 (1937).

[68] C. J. Malm, L. J. Tanghe and B. C. Laird, Ind. Eng. Chem., 38, 77 (1946).

as plasticisers instead of camphor. For the molding operation, heat and pressure or injection molding may be employed. In the preparation of lacquers, tricresyl phosphate frequently is used as the plasticizer. Such lacquers have particular value as insulating coatings and for protecting airplane fabrics. A mixed cellulose acetate butyrate (with 14–18% butyryl and 32–36% acetyl) is said to have even more desirable properties than cellulose acetate for these latter purposes. Cellulose acetate butyrates of 35 to 40% butyryl content are said to be particularly suitable for molding compositions.[69] Mixed esters of cellulose in which the acyl groups are lower fatty acids (acetic to butyric) and long-chain fatty acids (stearic, oleic) are prepared from the acyl halides using pyridine as catalyst.[70]

c. OTHER ESTERS

Benzoate, formate, cinnamate, phthalate and other esters of cellulose have been investigated but do not seem to have found commercial application.

An interesting type of ester is formed by the reaction of pentaacetyl-gluconyl chloride on mercerized cotton linters or cellulose acetate. By the use of triethylamine as catalyst, 0.45 mole of the acetylgluconyl groups are introduced per glucose unit. Mixed esters are obtained from a partially acetylated cellulose using pyridine as catalyst, and 0.75 acetylgluconyl groups are attached to each glucose unit. The mixed ester forms colorless, flexible, transparent films which are soluble in acetone and chloroform.[71]

Esterification of partially acetylated (acetone-soluble) cellulose acetates by dibasic acids leads to three types of derivatives: those (I) with cross linkages between cellulose chains, those (II) with unsubstituted carboxyl groups and those (III) with the second carboxyl group esterified with an alcohol.

$$
\begin{array}{ccccc}
& O & O & & O \\
& \| & \| & & \| \\
HC-O & C & (C)_n & C-O & CH, \\
& & & & HC-O-C & (C)_n-COOH, \\
| & & & | & | \\
\end{array}
$$

(I)   (II)

$$
\begin{array}{cc}
& O \\
& \| \\
HC-O-C- & (C)_n-COOR \\
| & \\
\end{array}
$$

(III)
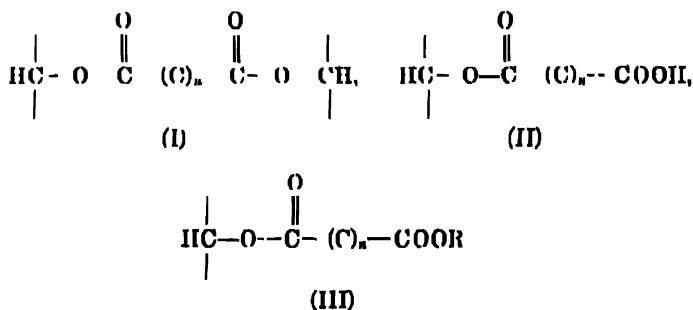
[69] C. R. Fordyce and L. W. A. Meyer, *Ind. Eng. Chem.*, **32**, 1053 (1940).

[70] G. W. Seymour and B. B. White, U. S. Patent 2,390,185, Dec. 4, 1945.

[71] M. L. Wolfrom and P. W. Morgan, *J. Am. Chem. Soc.*, **64**, 2026 (1942).

Such derivatives exhibit a wide range of solubilities. Cross-linking reduces the solubility, and the presence of free carboxyl groups (e.g., in the cellulose acetate acid phthalates) gives rise to soluble sodium and ammonium salts.[72]

Alkyl and particularly aryl isocyanates react with celluloses and partially acetylated celluloses, and as many as three carbamyl groups may be introduced into each glucose residue.

$$
\begin{array}{ccc}
\text{H—C—OH} & & \text{H—C—OC—NHC}_6\text{H}_5 \\
| & \xrightarrow[\text{pyridine}]{\text{C}_6\text{H}_5\text{NCO}} & | \\
\text{H—C—OCCH}_3 & & \text{H—C—OCCH}_3
\end{array}
$$
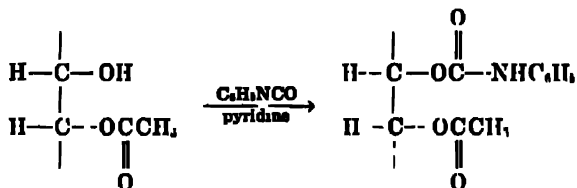
The carbamyl groups are resistant to acid and alkaline hydrolysis under conditions such that the acetyl groups are completely removed.[73]

Cross esterification of methylated celluloses (degree of substitution, 2.3) occurs as a result of treatment with oxalyl chloride in the presence of pyridine. These products form clear gels.[74]

E. Cellulose Ethers. The hydroxyl groups of cellulose are etherified by the reaction of alkali cellulose with alkyl halides or alkyl sulfates in a manner similar to that for the simple sugars (p. 345). The reaction is heterogeneous in character because of the insolubility of the cellulose in the alkylating medium. Partially alkylated products are obtained unless considerable care is taken to insure complete etherification, and degradation of the cellulose chains commonly occurs. An ingenious method has been devised by Mahoney and Purves[75] for investigating the distribution of the alkyl groups in cellulose ethers. The number of free primary alcoholic groups is measured by reaction with p-toluenesulfonyl chloride and replacement of the tosyloxy groups by iodine (p. 171). Determinations of the number of unsubstituted glycol groups in the cellulose ether and in its hydrolytic products are made by periodic acid or lead tetraacetate oxidation. A commercial ethylcellulose with 2.48 ethoxyl groups and 0.52 hydroxyl groups per glucose unit was oxidized with lead tetraacetate and shown to have 0.01 free glycol groups at carbons 2 and 3. The extent of oxidation by lead tetraacetate of the products of acid hydrolysis (anomeric hydroxyl now unsubstituted) corresponds to 0.13 to 0.15 unsubstituted hydroxyls in position 2. Since the tosylation procedure reveals the presence of 0.12 unsubstituted primary hydroxyl groups, the number of unsubsti-

[72] C. J. Malm and C. R. Fordyce, *Ind. Eng. Chem.*, **32**, 405 (1940).

[73] W. M. Hearon, G. D. Hiatt and C. R. Fordyce, *J. Am. Chem. Soc.*, **65**, 829, 833 (1943).

[74] R. Signer and P. v. Tavel, *Helv. Chim. Acta*, **26**, 1972 (1943).

[75] J. F. Mahoney and C. B. Purves, *J. Am. Chem. Soc.*, **64**, 9, 15 (1942).

tuted hydroxyls at carbon 3 is $0.52 - 0.13 - 0.12 = 0.27$. As the number of free glycol units at carbons 2 and 3 is only 0.01, the ethoxyl groups must be distributed between the various residues rather than accumulated on a few.

Another method of studying the distribution of substituents involves the measurement of the amount of cellobiose octaacetate produced by acetolysis of partially methylated cellulose.[76] In the early stages of etherification, multiple substitution of exposed residues occurs, but when a degree of substitution of 1.5 has been attained the crystalline areas are penetrated strongly.

A microscopic study of the changes taking place in the fibers of alkali cellulose (prepared from cellulose linters) during benzylation illustrates the difficulties which arise from the heterogeneous character of the reaction.[77] The benzylation reaction involves three phases, one solid (alkali cellulose) and two liquid (sodium hydroxide solution and benzyl chloride). A proper balance must be established between the rate of etherification and the diffusion into the fiber structure. When the diffusion is too slow, complete benzylation of the outer layers takes place and the inner layers are kept from reacting by an impervious film of benzylcellulose. Inasmuch as the chemical reaction has a larger temperature coefficient than the rate of diffusion, complete substitution is favored by the use of the lowest possible temperatures. Methyl and ethyl chlorides diffuse readily into the swollen fiber and therefore the reaction is much less difficult than with larger groups.

a. METHYL AND ETHYL ETHERS

The methyl and ethyl ethers are prepared by treatment of the alkali cellulose or cellulose acetate with the alkyl sulfate or chloride and alkali, and usually a number of treatments are necessary. To obtain complete methylation without degradation, it is necessary to work at low temperatures and in nitrogen (see p. 535).

$$-\overset{\vert}{\underset{\vert}{C}} - O \quad H \xrightarrow{\text{(CH}_3)_2\text{SO}_4} \overset{\vert}{\underset{\vert}{C}} - OCH_3$$
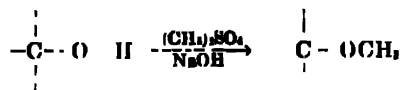
It is difficult to achieve complete methylation (45.57% OCH_3).[78] The use of a quaternary ammonium hydroxide as the base improves the method, particularly when water-soluble products of low alkyl content are desired.[79] The solubility of these products varies markedly with the alkoxyl content. Although the original cellulose is insoluble in water and organic solvents,

[76] T. Lieser and R. Jaks, *Ann.*, **548**, 204 (1941).

[77] E. J. Lorand and E. A. Georgi, *J. Am. Chem. Soc.*, **59**, 1166 (1937).

[78] See discussion by: G. G. Johnston, *J. Am. Chem. Soc.*, **63**, 1043 (1941).

[79] L. H. Bock, *Ind. Eng. Chem.*, **29**, 985 (1937).

the product becomes soluble in cold water as the methoxyl content approaches 1.5 groups per glucose unit; at still higher methoxyl contents, the substance becomes water insoluble and more soluble in organic solvents (pyridine, chloroform, etc.). Interesting enough, the material soluble in cold water is insoluble in hot water and precipitates from solutions which are heated although it redissolves in cold water. This phenomenon is ascribed to the formation of hydrates which dissociate at higher temperatures. The increase in water solubility as a result of *partial* methylation may be due to the disorienting effect of the alkoxyl groups. Thus, the methoxyl groups may prevent the association of neighboring cellulose chains by their steric effect (i.e., they are larger than hydroxyl groups), but at the same time there will still be enough hydroxyl groups to confer water solubility[79,80]

The methylated celluloses have been of principal value for the elucidation of the structure of cellulose, but water-soluble methylcelluloses are used for the preparation of printing inks for textiles. Methylcelluloses have good emulsifying properties which should be of value for such products as soaps, cleaners, waxes, etc. Their use as textile sizes and finishes for textiles, leather and paper has been suggested. The ethylcelluloses are of considerable industrial importance as components of lacquers and plastics.

b. OTHER ETHERS

Other ether derivatives of cellulose are known, but, with the exceptions of benzylcellulose and carboxymethylcellulose, they are not of industrial importance although several have interesting properties. Benzylcellulose, made by the reaction of benzyl chloride on alkali cellulose, is less hydrophilic than the methylcellulose and formerly found application in injection molding but its low softening point and extreme sensitivity to light and heat have caused it to be largely replaced by other cellulose derivatives. Glycolcellulose (hydroxyethylcellulose), made by the action of ethylene oxide on alkali cellulose, is soluble in water and insoluble in organic solvents.[81] The substance presumably is the monoether of ethylene glycol, and upon acetylation three acetyl groups are introduced for each glucose residue.

$$
\begin{array}{c}
| \\
HC-OH
\end{array}
+ CH_2--CH_2 - \rightarrow
\begin{array}{c}
| \\
HC-OCH_2-CH_2OH
\end{array}
\xrightarrow{(Ac)_2O}
$$

$$HC-OCH_2\cdot CH_2OAc$$
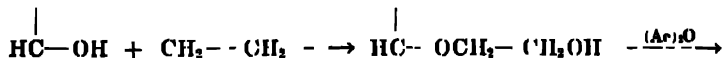
[79] D. Traill, *J. Soc. Chem. Ind.*, **53**, 337 (1934); E. Heymann, *Trans. Faraday Soc.*, **31**, 846 (1935); **32**, 402 (1936).

[81] See: P. Schorigin and J. Rymaschowskaja, *Ber.*, **66**, 1014 (1933); A. W. Schorger and M. J. Shoemaker, *Ind. Eng. Chem.*, **29**, 114 (1937).

Carboxymethylcellulose is formed by reaction between monochloroacetic acid and alkali cellulose.[82]

$$\underset{|}{\text{HC}}\text{—OH} + \text{ClCH}_2\text{COOH} \longrightarrow \underset{|}{-\text{C}}\text{—O—CH}_2\text{COOH}$$
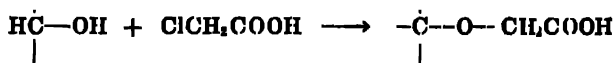
Because of the presence of the carboxyl groups, the ether is soluble in alkalies and water and insoluble in organic solvents. The action of phosphorus triiodide severs the ether linkage, and cellulose and glycolic acid are liberated. The dissociation constant of the material has a value of 5×10^{-5} and is similar to that of other fairly strong organic acids.

Cyanoethylcellulose of a high degree of substitution is made by treatment of cellulose (*D.P.* 350 to 400) with a large excess of acrylonitrile in the presence of alkali.[83]

Monotrityl derivatives[7][8] are formed from cellulose by treatment with triphenylmethyl chloride; as with the simple sugars (p. 348), the primary alcoholic groups are preferentially etherified.

F. Methylene Derivatives.[84] Reaction of cellulose with the *n*-propyl acetal of formaldehyde $[(\text{C}_3\text{H}_7\text{O})_2\text{CH}_2]$ in the presence of acetic anhydride results in the introduction of one methylene group for each pair of glucose residues (8% CH_2O). Since acetic anhydride liberates formaldehyde from the propyl acetal, the reaction probably resembles that of the simple sugars with aldehydes and ketones (p. 222), which results in the formation of an acetal or ketal in which two hydroxyls are involved. The number of methylene groups per hydroxyl group may be increased considerably (17.2% CH_2O) by carrying out the reaction with $((\text{ClCH}_2)_2\text{SO}_2$ and similar esters. The products exhibit some unusual properties that make them of interest. As the methylene content increases, the products have less tendency to swell in alkaline solutions and become less soluble in cuprammonium solution. Highly substituted products are very unreactive under alkaline conditions but still are susceptible to hydrolysis by acids. Fibers made from such material are very brittle.

These unusual properties probably result from the formation of methylene linkages (CH_2 -) between neighboring cellulose chains rather than within the same glucose residue.

[82] J. K. Chowdhury, *Biochem. Z.*, *148*, 76 (1924); W. L. Barnett, *J. Soc. Chem Ind*, *40*, 253 (1921); C. J. Brown and A. A. Houghton, *J. Soc. Chem. Ind.*, *60*, 254 (1941). Possible uses of this material are described by C B Hollabaugh, L H. Burt and A. P. Walsh, *Ind Eng Chem*, *37*, 913 (1945)

[83] R. C. Houtz, U. S. Patent, 2,375,847, May 15, 1945

[84] M. Schenk, *Helv. Chim Acta*, *15*, 1088 (1932); F. Wood, *J Soc. Chem. Ind.*, *50*, 411 (1931); P. Schorigin and J Rymaschewskaja, *Cellulosechem.*, *14*, 81 (1933); K. Gotze and A. Reiff, *Chem. Abst*, *37*, 2570 (1943).

$$\text{H}-\overset{\displaystyle |}{\underset{\displaystyle |}{\text{C}}}-\text{OH} + \text{CH}_2\text{O} + \text{HO}-\overset{\displaystyle |}{\underset{\displaystyle |}{\text{C}}}-\text{H} \longrightarrow \text{H}-\overset{\displaystyle |}{\underset{\displaystyle |}{\text{C}}}-\text{O}-\text{CH}_2\text{O}-\overset{\displaystyle |}{\underset{\displaystyle |}{\text{C}}}-\text{H}$$
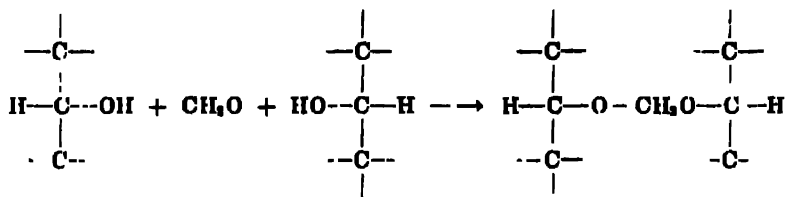
Such cross linkages would stabilize the position of the chains, prevent swelling and decrease the elasticity.

G. Hydrolysis by Enzymes.[35] It is common knowledge that wood and other cellulosic material "decay," but the action of enzymes and microorganisms on cellulose has not received investigation commensurate with the importance of the subject. Controlled enzymic hydrolysis or fermentation of waste cellulosic materials, in the future, may provide new and cheap sources of sugars and organic chemicals. The natural decomposition of cellulose products is due to bacterial and fungal action, but a discussion of the action of microorganisms lies outside the scope of the present work.[36]

Because of the insolubility of cellulose, it seems probable that the action of microorganisms takes place by the secretion of enzymes (cellulases) onto the surface of the substrate material. Cellulases seem to be fairly widely distributed in fungal and bacterial emulsins and in the digestive juices of snails, crustacea and certain fish.

Pringsheim reports that the hydrolysis of cellulose by enzymes of thermophilic bacteria proceeds through the intermediate formation of cellobiose, and that a cellobiase (β-glucosidase) is also present which continues the hydrolysis to glucose. It is claimed that the cellulase is more stable to higher temperatures than the β-glucosidase, and by conducting the hydrolysis at 67°C., an accumulation of cellobiose takes place.[37]

The action of snail cellulase on various types of celluloses has received attention from Karrer and associates.[38] By repeated enzymic treatment, it is possible to obtain degrees of hydrolysis of cuprammonium and viscose rayon as high as 95 to 96%. Under similar conditions, Helferich and Goerdeler[39] report 62% saccharification of cellophane as a result of a

[35] P. Karrer, "Polymere Kohlenhydrate," p. 108, 211; Akademische Verlagsgesellschaft, Leipzig (1925).

[36] For information on this phase of the subject, the following references are suggested: A. G. Norman and W. H. Fuller, *Advances in Enzymology*, 2, 239 (1942). A. C Thaysen and H. J. Bunker, "The Microbiology of Cellulose, Hemicelluloses, Pectin and Gums," Oxford University Press, London (1927). S. A. Waksman, *in* "Wood Chemistry," (L. E. Wise, Editor), p. 828; Reinhold, New York (1944).

[37] H. Pringsheim and W. Kusenack, *Z. physiol. Chem.*, 137, 265 (1924); H. Pringsheim, *ibid.*, 78, 266 (1912).

[38] P. Karrer *et al.*, *Helv. Chim. Acta*, 9, 893 (1926); 11, 229 (1928)

[39] B. Helferich and J. Goerdeler, *Ber. Verhandl. sächs. Akad. Wiss Leipzig, Math phys. Klasse*, 92, 75 (1940).

single treatment. Native cotton cellulose and wood are much more resistant to enzymic hydrolysis than are the degraded celluloses. Since the reaction is heterogeneous in character, it is possible that one of the main variables is the surface area or relative degree of crystallinity of the substrate. This factor has never been given adequate consideration, and it is probable that the differences in the relative crystallinity may to a considerable extent explain the great differences observed between the various cellulose types. The action of cellulases on wood has been studied in particular by Ploetz.[90]

In a study of the action of the cellulase and cellobiase (β-glucosidase) of *Aspergillus oryzae* emulsin on a series of cellodextrins, it was shown that the two enzymes can be separated by preferential adsorption on aluminum hydroxide and that the cellulase can hydrolyze cellulose and degradation products down to a molecular weight of about 1000. On the other hand, the β-glucosidase can hydrolyze cellobiose and cellohexaose but not the cellodextrins with molecular weights greater than about 1000.[91]

Both cellulose and lichenin are usually degraded by the action of the crude enzyme preparations of the types described above. The action of *Aspergillus oryzae* emulsins on these two substrates seems to be due to the presence of two distinct enzymes which may be separated by fractional precipitation with alcohol and ether and which have different optimal acidities.[92]

[90] T. Ploetz, *Ber.*, 72, 1885 (1939), 73, 57, 61, 71 (1940)

[91] W. Grassmann, L. Zechmeister, G. Tóth and R. Stadler, *Ann.*, 503, 107 (1933)

[92] K. Freudenberg and T. Ploetz, *Z. physiol. Chem.*, 259, 19 (1939)

THE STARCHES AND STARCH SUBSTANCES

1. Introduction

Both cellulose and starch are glucose polymers and frequently are found in association, but they exhibit widely different properties and have different biological functions. The role of cellulose is that of a structural substance, but starch acts as a reserve material. During the growing period of the plant, the glucose is stored as fine granules of starch in the seeds, roots or fruits. In periods of active plant growth, particularly in the spring, the starch is converted to soluble sugar and transported to the position where it is required for transformation to cellulose or other products.

In the portions of the plant in which starch is stored, the starch content may be very great. Thus, some seeds and grains may consist of as much as 70% of starch. The tubers and roots of many plants and the pithy stems of certain palms contain smaller quantities which may be as great as 30%. The starches of most interest from the industrial standpoint are derived from corn, potatoes and tapioca.[1] Others of less commercial interest are obtained from wheat, sago, sweet potatoes[2] and rice. Since tapioca (cassava) starch is produced from an East Indian plant (*Manihot* species) and must be imported, there is considerable interest in developing starches of similar properties from waxy grains such as sorghum and maize. Waxy maize starch resembles tapioca starch in many of its properties and has been made on a commercial scale.[3] A substance closely related to starch and called glycogen is found in animal tissues, particularly in liver and muscle tissues, and serves as a reserve polysaccharide.

The term starch covers a number of related substances having different structures and molecular weights. In the present discussion, it is used when reference is made to properties or reactions common to all of the constituents or when no attempt has been made to separate the various starch substances. It might be preferable to speak of starches rather than starch, since not only is the product obtained from a single source inhomogeneous, but the relative proportion of the various constituents varies according to their origin. Because of their technological and biological importance,

[1] For a comprehensive discussion of the starch industry from the economic and trade viewpoint see: "Starch, Dextrines and Related Products," U. S. Tariff Commission Report no. 138 (1940); Superintendent of Documents, Washington, D. C.

[2] H. S. Paine, F. H. Thurber and R. T. Balch, *Ind. Eng Chem.*, **30**, 1330 (1938); F. H. Thurber, *ibid.*, **25**, 565 (1933).

[3] See: H. H. Schopmeyer, G. E. Felton and C. L. Ford, *Ind Eng. Chem.*, **35**, 1169 (1943).

the starch substances have received much study, but more investigation is required before their structures may be considered as established.

The identification of starches of different types is accomplished by an examination of the size and shape of the granules, the temperature of gelatinization, the degree of polarization (under crossed nicols), the rate of swelling (in the presence of agents such as chloral hydrate or chromic acid) and the extent of coloration by iodine. Reichert[4] has suggested a systematic scheme of identification based on these properties. A more recently developed property which may be of value for this purpose is the amount of linear and branched chain material present (amylose and amylopectin) (see below).

2. Composition

A. General Composition of Starch Granules. Native starch is found as microscopic, anisotropic granules exhibiting characteristic differences in form; its source often may be determined by an examination of the shape and size of the granules (see Fig. 1). The shape may be oval, spherical or irregular, and the size is usually in the range 0.002 to 0.15 mm. Stratified layers are visible particularly after the grains are swollen in hot water. Intact starch grains are insoluble in cold water, even after long soaking; but after the outer membranes are broken by crushing or grinding, the material swells in water. The effect of severe grinding may be to break intermolecular linkages, for the amount of material soluble in cold water and the reducing power increase progressively as the grinding process is continued.[5]

Hot water produces swelling of intact granules, and at sufficiently high temperatures, the granules burst and form viscous solutions or gels. When such solutions are cooled, rigid gels may be formed by the crystallization of the dissolved or dispersed starch substances as networks occluding much solution. Other solvents also act as gelatinizing media; to be included among these solvents are liquid ammonia, liquid HCN, formamide and formic acid. Alkalies and nitrogenous bases exert a dispersing action even at room temperature, and salts in the order of the Hofmeister series act similarly.

In addition to the carbohydrate content, the starch grains contain minor constituents. Some, although present in small quantity, have been assigned an important part in the explanation of certain properties. Much of the water content of the granules forms a part of the crystalline structure, for

[4] E. T. Reichert, *Carnegie Inst Publ* , No. 173 (1913); F. D. Armitage, *Ind. Chemist*, *19*, 383 (1943). J A. Radley, "Starch and Its Derivatives," p. 205, Van Nostrand, New York (1940). O. R. Trubell *in* "Chemistry and Industry of Starch," R. W. Kerr, Editor, Academic Press, New York (1944).

[5] L. H. Lampitt, C. H. F. Fuller and N. Goldenberg, *J. Soc. Chem. Ind.*, *60*, 1, 25, 301 (1941).

Fig 1 Microphotographs of starch granules ($\times$ 200)

A Corn starch
B Potato starch
C Wheat starch
D Tapioca starch
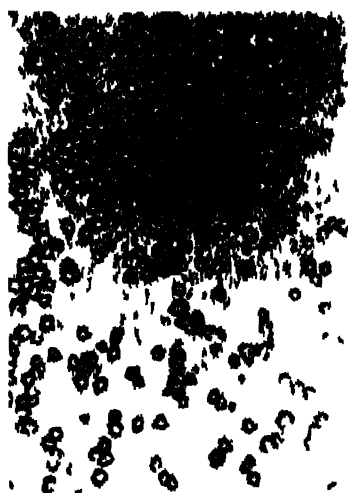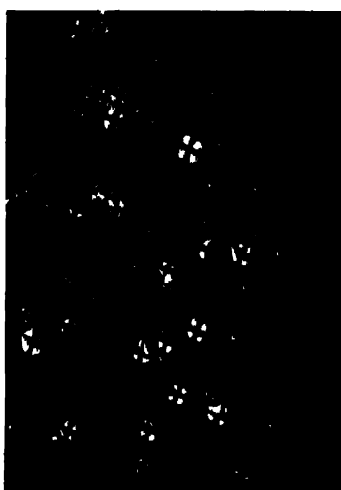
FIG. 1 (Continued)

F Rice starch
F' Waxy maize starch
G Sweet potato starch
H Tapioca starch in polarized light
(Reproduced from "Chemistry and Industry of Starch," R. W. Kerr, Editor, Academic Press 1944.)

when it is removed, the X-ray diffraction pattern changes from a crystalline to an amorphous diagram. Various higher fatty acids are found associated with the carbohydrate in amounts occasionally higher than 0.6%.[6] Although Taylor and Lehrman have considered that the fatty acids are combined with the starch, Schoch[7] has shown that the acids may be extracted by water-soluble fat solvents such as methanol and that they are merely adsorbed on the carbohydrate. For scientific investigations, it is recommended that raw starches be purified by extraction with boiling 80% methanol.

A small amount of phosphorus is found in the starch grain (0.01 to 0.2% P). The combined phosphorus has been considered, particularly by Samec and his associates,[8] to play an important part in the structure of the starch molecule. Other workers, however, ascribe no significance to this constituent. The phosphorus of corn, wheat and rice starches is removed by repeated extraction with warm water and alcohol, and glycerol monophosphate has been identified in the extracts.[9] Other starches, of which potato starch may be considered as representative, contain esterified phosphorus. On hydrolysis with acids or enzymes, glucose 6-phosphate is formed.[9] Potato and similar starches, in contrast to the cereal starches, exhibit a close correlation between the phosphorus content and the conductivity, hydrogen ion concentration and titratable acidity.[8] This distinction between cereal and root starches is also shown in the extractability of the phosphorus constituents by the action of boiling 80% dioxane. The phosphorus constituents of wheat starch are almost completely removed, but those of potato starch are only affected to a minor degree.[7] The esterified phosphate is removed by heating aqueous starch solutions under pressure as well as by the action of enzymes. Although certain characteristics such as the pasting and acidic properties are affected by this treatment, they are restored by rephosphorylation and become accentuated upon increase of the phosphorus content above the normal quantities.[8] Small quantities of silicic acid (<0.07%) and nitrogenous substances also are associated with the starch granule.

B. Amylose and Amylopectin. Starch granules consist of two principal types of carbohydrates: a straight-chain, polymerized maltose and a branched-chain, polymerized maltose. According to K. H. Meyer,[10] the straight-chain molecules are to be called amylose and the branched-chain

[6] T. C. Taylor and L. Lehrman, *J. Am. Chem. Soc*, **48**, 1739 (1926); L. Lehrman and E. A. Kabat, *ibid*, **59**, 1050 (1937); J. W. Evans and D. R. Briggs, *Cereal Chem.*, **18**, 443, 465 (1941).

[7] T. J. Schoch, *J. Am. Chem. Soc.*, **64**, 2144 (1942).

[8] See: M. Samec, *Chem.-Ztg.*, **63**, 353 (1939); M. Samec and M. Blinc, *Kolloid-Beihefte*, **47**, 371 (1938); M. Samec and L. Zagar, *Wien. Chem. Ztg.*, **46**, 25 (1943).

[9] T. Posternak, *Helv. Chim. Acta*, **18**, 1351 (1935).

[10] K. H. Meyer, *Naturwissenschaften*, **28**, 397 (1940).

molecules, amylopectin. These names had been used previously to designate starch factions obtained by a variety of methods, some of which are described below. Unfortunately few, if any, of these products called "amylose' and "amylopectin" conform to the definition of Meyer. In a few cases, it is reasonably certain that the reported "amylose" fraction resembles Meyer's amylopectin more than his amylose. Many of the products have been impure mixtures, probably scarcely purer than the original starch. In the subsequent discussion, it will be shown that effective separation of the starch components may be obtained by treatment of starch sols with butanol and certain other polar organic substances; one fraction is precipitated by the alcohol, and the other remains in solution. The butanol-precipitated material appears to correspond well with the Meyer definition for amylose; the unprecipitated fraction is the amylopectin of Meyer. Hence in the subsequent discussion, the terms amylose and amylopectin will be used to describe components pure enough to agree with the Meyer definition. Materials of doubtful purity will be indicated by quotation marks around the name of "amylose" and "amylopectin."

Many other names for starch fractions are found in the literature. Some approximate equivalents for "amylose" are β-amylose, starch cellulose, and amyloamylose; synonyms for "amylopectin" are α-amylose, erythro-amylose and granulose. Some inversion of these terms is found in the literature.

According to the original definition of Maquenne,[11] "amylopectin" is the portion of the starch granule which is responsible for the formation of starch gels and which is hydrolyzed by malt enzymes to dextrins. Most investigators have used "amylose" to describe the material leached from starch granules by hot water. For the separation of the components, Samec employs electrophoretic separation of starch which has been dissolved in superheated water. "Amylopectin" migrates and precipitates at the anode whereas the "amylose" remains in solution.[12] There is some doubt as to whether these fractions are related to the products, with corresponding names, obtained by the solubility method. Another procedure involves the freezing of a starch paste previously heated to 130°C. The frozen mass is allowed to thaw, and the "amylose" is extracted with hot water (60°C.).[13] The preferential adsorption of amylose on cellulose (cotton) has also been employed for the separation.[14]

An important contribution to the problem of obtaining pure starch

[11] L. Maquenne and E. Roux, *Compt. rend.*, *140*, 1303 (1905).

[12] M. Samec, *et al.*, *Kolloid-Beihefte*, *18*, 281 (1920); *13*, 272 (1921); T. C. Taylor and H. A. Iddles, *Ind Eng. Chem* , *18*, 713 (1926); R. M. Hixon and V. D. Martin, *Ind. Eng. Chem., Anal. Ed.*, *11*, 395 (1939).

[13] A. R. Ling and D. R Nanji, *J. Chem. Soc.*, *123*, 2666 (1923).

[14] C. Tanret, *Compt. rend.*, *158*, 1353 (1914); E. Pacsu and J. W. Mullen, *J. Am. Chem. Soc.*, *63*, 1168 (1941); M. Samec, *Ber.*, *73A*, 88 (1940).

fractions has been made by T. J. Schoch. When a starch dispersion made by autoclaving starches and water is saturated with butanol, a crystalline precipitate forms.[15] The damp, crystalline material dissolves easily in water. This material is crystalline amylose. From the mother liquors, amorphous amylopectin is precipitated by the addition of water-soluble alcohols.

Other polar organic materials may be used in the place of butyl alcohol.[16] A comparison of various alcohols for use in the separation of the starch fractions resulted in the following order of preference:

Preferred: *n*-Amyl alcohol and Pentasol.

Excellent: Hexyl alcohol, 2-ethyl-1-butanol, lauryl alcohol, cyclo hexanol, 30% isopropyl alcohol.

Good: *n*-Butyl alcohol, 3-pentanol, 4-methyl-2-pentanol, *d*,*l*-borneol, α-terpineol, *n*-propyl alcohol (20%).

Fair: Isobutyl alcohol, *sec.*-butyl alcohol, 2-methyl-1-butanol, isoamyl alcohol, *tert.*-amyl alcohol, menthol, 40% *n*-propyl alcohol.

Poor: Ethyl alcohol (30%), benzyl alcohol.

Many other polar organic substances may be used for this purpose Among these are the fatty acids, certain nitroparaffins and thymol.[17]

The precipitating action of fatty acids on amylose is of particular interest because of the natural occurrence of fatty acids in starches. Unless the fatty acids are removed from starches by extraction with polar organic solvents (e.g., methanol or dioxane), erroneous results may be obtained because of the removal of amylose as an insoluble complex with fatty acids.[18] As shown by Wilson, Schoch and Hudson, the "γ"-amylose (also known as amylocellulose and amylohemicellulose) described in the literature undoubtedly is a fatty acid-amylose complex. This material separates as an insoluble precipitate during the enzymic hydrolysis of unpurified starches. Corn starch has a very low content of phosphate groups. When dispersed and placed in an electrophoretic cell, a fraction of corn starch migrates. This fraction appears to be a portion of the amylose that has been made polar by the adsorption of the natural fatty acids.

[15] T. J. Schoch, *J. Am. Chem. Soc.*, *64*, 2957 (1942); R. W. Kerr and G. M. Severson, *ibid.*, *65*, 193 (1943), E. J. Wilson, Jr., T. J Schoch and C. S. Hudson, *ibid.*, *65*, 1380 (1943); E Wiegel, *Z. physik. Chem.*, *188A*, 137 (1941); F Wiegel, *Kolloid-Z*, *102*, 145 (1943).

[16] T. J. Schoch, *Advances in Carbohydrate Chem.*, *1*, 247 (1945).

[17] T. J. Schoch and C B. Williams, *J. Am. Chem. Soc.*, *66*, 1232 (1944); R. L. Whist ler and G. E. Hilbert, *ibid.*, *67*, 1161 (1945); W. N. Haworth, S Peat and P. E. Sagrott, *Nature*, *157*, 19 (1946).

[18] T. J. Schoch and C. B. Williams, *J. Am. Chem. Soc.*, *66*, 1232 (1944); F J. Wilson, Jr , T J. Schoch and C. S. Hudson, *ibid.*, *65*, 1380 (1943).

In addition to making crystalline amyloses available for starch studies, the precipitation procedure also provides a means for the analysis of starches, for the precipitation of amylose appears to be nearly quantitative. Of the starches analyzed by this method, the proportions of amylose vary from 0% in waxy maize to a maximum of 35% in lily bulb starches. Ordinary corn and potato starches contain 22% of the amylose constituent.[19] It should be noted that the amylose fractions from all starches are not identical. This difference is shown in the solubility and crystal form; the variations in these properties probably are to be ascribed to differences in the molecular weights of the amylose fractions rather than to differences in structure.
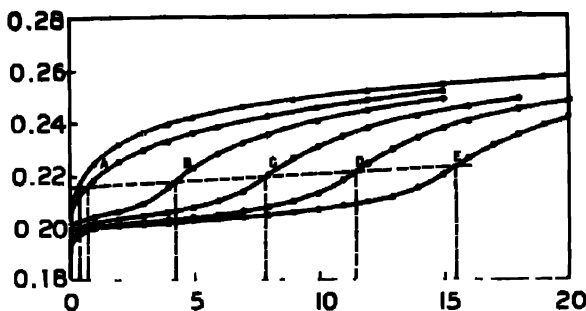
Fig. 2 Iodine activity in amylose and amylopectin solutions.
Curve A, 0.01 g. of amylopectin from potato starch; Curve B, 0.0075 g of amylopectin from potato starch plus 0 0025 g. of "crystalline amylose;" Curve C, 0.0050 g. of amylopectin from potato starch plus 0.0050 g. of "crystalline amylose;" Curve D, 0.0025 g of amylopectin from potato starch plus 0.0075 g of "crystalline amylose;" Curve E, 0.01 g. of "crystalline amylose " The uppermost curve is for a 0.05 N KI solution Ordinate: E.M.F. against normal calomel electrode, 0.18, 0.20, 0.22, 0.24, 0 26, 0 28. Abscissa: cubic centimeters of 0.001 N iodine solution, 0, 5, 10, 15, 20.
(Reproduced from "Chemistry and Industry of Starch," R. W. Kerr, Editor.)

The two starch fractions exhibit different behaviors in iodine solutions, and an important method of analysis of starch constituents has been developed by the use of iodine titrations.[20] If successive portions of iodine are added to amylose solutions, the iodine activity (as measured from the E.M.F. of the cell: Pt | I_2, KI | calomel) remains fairly constant until characteristic amounts of iodine have been added; thereafter, the activity increases as more iodine is added (see Fig. 2, Curve E). This behavior is best interpreted as a removal of the iodine from the solution through the

[19] These values may need some revision in view of improvements in the method of analysis. "Pentasol," a mixture of amyl alcohols, gives a value of 28% amylose for corn starch. The method of iodine adsorption, discussed later, gives low values in the presence of fatty acids.

[20] F L. Bates, D. French and R. E Rundle, *J. Am. Chem. Soc*, *65*, 142 (1943)

formation of a starch-iodine complex. In contrast to the behavior of the amylose fraction, the addition of iodine to amylopectin solutions results in a continuous increase in the activity of the iodine proportional to the amount of iodine added (Curve A). Mixtures of amylopectin and amylose behave similarly to amylose alone except that the initial period of stationary potential is less than for pure amylose (Curves B, C, D). The amount of iodine bound in the initial period is a function of the amount of amylose present and may be used for its estimation. The application of this method to the determination of the amylose content leads to the following results: barley, corn, sorghum, and rice starches of the waxy variety and glycogen contain no amylose; lily bulb starch contains the most amylose (34%); and in between these limits are the tapioca and rice starches (17%), the wheat, popcorn, potato, corn, and banana (21-24%), and the sago starch (27%). In most instances, these results agree well with the results of the butanol-precipitation method.

Another quantitative method suggested for the estimation of the amylose and amylopectin contents of starches depends on measurement of the absorption spectra of the complexes formed by the two components with iodine.[21] An analysis of starch from wrinkle-seeded peas of the garden type shows that this material has an unusually large amount of amylose, 75%.[22] This starch should be particularly interesting because of its high amylose content. The extent of conversion of starches by enzymes (β-amylases) also has been applied as a measure of the amounts of the two starch constituents. By the latter method, it has been shown that starch granules from growing potato sprouts may contain much more amylose than those from leaves and tubers.[23]

The two main starch fractions are characterized by fairly sharp differences in a number of properties. Amyloses give a deep blue color with iodine, adsorb fairly large quantities of iodine from solution, and are completely adsorbed by cellulose. The solutions yield gels that are quite unstable and deposit crystalline material. In contrast, amylopectins give red to purple colors with iodine, adsorb less iodine and in a different manner, and are not adsorbed by cellulose. Solutions of amylopectin are fairly stable even when saturated with butanol. Amyloses have appreciably lower molecular weights than amylopectins and are hydrolyzed to a much greater extent by β-amylases. As will be shown later, the amylose fractions have a linear structure analogous to cellulose, whereas the amylopectins are

[21] R. M. McCready and W. Z. Hassid, *J. Am. Chem. Soc.*, **65**, 1154 (1943); R. R. Baldwin, R. S. Bear and R. E. Rundle, *ibid.*, **66**, 111 (1944); R. W. Kerr and O. R. Trubell, *Paper Trade J.*, **117**, No. 15, 25 (1943).

[22] J. P. Nielsen and P. C. Gleason, *Ind. Eng. Chem., Anal. Ed.*, **17**, 131 (1945); G. E. Hilbert and M. M. McMasters, *J. Biol. Chem.*, **162**, 229 (1946).

[23] K. H. Meyer and P. Heinrich, *Helv. Chim. Acta*, **26**, 1038, 1630 (1942).

highly branched. Amylopectins from potato and tapioca starch frequently are associated with phosphate groups, which may be responsible for their electrophoretic behavior Amyloses give X-ray diagrams indicating a high degree of crystallinity, whereas the diagrams given by amylopectins u ually are of the amorphous type.

There is little doubt but that the amylose and amylopectin fractions are heterc geneous both in regard to the molecular size of the molecules as well as in the degree of branching. Such a concept explains the known differences between starches which have quite different properties but which have about the same relative amounts of the two main fractic ns, e.g., corn starch and tapioca starch. There is not much experimental evidence to suppc rt this view, but Kerr[24] has shown that corn amylose can be fractionated into materials having different intrinsic viscosities.

C. Soluble Starches. The pasting and gelatinizing properties of starches may be altered to give modified starches which dissolve in hot water with the formation of limpid solutions. These "soluble starches" are produced by the action of oxidizing or hydrolytic agents on raw starches. Many such products are described, but the best known are probably the Lintner and the Zulkowsky soluble starches. The Lintner method[25] involves the treatment of raw starch with 7.5% hydrochloric acid for seven days at room temperature. Zulkowsky,[26] however, heated the starch with glycerol at 190°C.

Many commercial soluble starches are made by the treatment of starches with acids at temperatures below the gelatinization point. These products are the "thin-boiling" starches. Other similar products are made by the oxidation of starches (see Oxidation products, below). The term "soluble starches" is somewhat inept because the products will not dissolve in cold water and because gelation of the solutions will take place, although the carbohydrate concentration that is necessary for gelation is higher than for unmodified starches.

D. Chemical Evidence for Structure. Carbon-hydrc gen and molecular weight determinations on whole starch lead to the formula $(C_6H_{10}O_5)x$. Acid hydrolysis produces D-glucose, and this process, employed in the commercial preparation of the sugar (Chapter III), yields more than 90% of the crystalline sugar. By the action of acetyl bromide or HBr in acetic acid, starch is converted to heptaacetylmaltosyl bromide in yields of 37 to 45%.[27] Maltose, in yields as high as 70-80%, is also formed by enzymic hydrolysis of corn and potato starches. Each of the $C_6H_{10}O_5$ units has three

[24] R. W. Kerr, *Cereal Chem.*, *7*, 377 (1945).

[25] C. Lintner, *J. prakt. Chem.*, [2] *34*, 378 (1886).

[26] K. Zulkowsky, *Ber.*, *13*, 1395 (1880).

[27] K. Freudenberg and K. Soff, *Ber.*, *69*, 1252 (1936).

unsubstituted hydroxyl groups as is shown by the formation of triesters and triethers such as the triacetyl and trimethylstarches.

The above evidence shows that starch is a polymer of glucose in which the glucose units are combined mainly through 1,4′ glucosidic bonds having the α-configuration (maltose type). This structure is confirmed by the isolation in yields of 80 to 90% of 2,3,6-trimethylglucose by the acid hydrolysis of methylated starch.[28] The high dextrorotation of both starch and maltose and the study of the kinetics of the hydrolysis are in agreement with a structure mainly based on 1,4′ glucosidic linkages with an α-configuration.[29] Methylated tri- and tetrasaccharides, with the maltose type of structure, are obtained by the methylation and distillation of the products of the acetolysis of starch.[30] From the above evidence, the basic structure of the starches may be formulated as a chain of maltose units:

Maltose residue
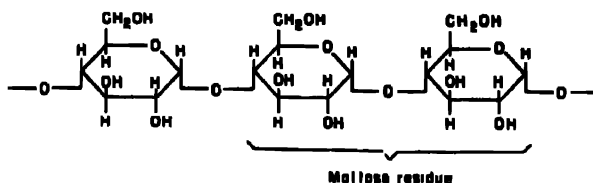
As will be shown, this structure best represents the amylose fraction of starches. The structure of the amylopectin is more complicated and may be derived from the above formula by the attachment of side chains through 1,6′ glucosidic bonds.

E. Molecular Weight of Starches.[31] In spite of many efforts to measure the molecular weights of starches, there is considerable disagreement in the results of different investigators. The origin of the differences arises to a considerable degree in the difficulty in bringing the starch fractions into solution without concurrent degradation and in the employment of inhomogeneous fractions. Although the amylose may go into solution readily, crystallization ("retrogradation") takes place fairly rapidly. For methods depending on the counting of the particles or ions (e.g., osmotic pressure), the amylopectin fraction may present difficulties because of the phosphate content which, although small, provides ionizable hydrogen atoms.

Osmotic Pressure Method. Samec[31] reports a molecular weight for potato

[28] J. C. Irvine and J. Macdonald, *J. Chem. Soc.*, 1502 (1926); E. L. Hirst and G. T. Young, *ibid.*, 951 (1939).

[29] K. Freudenberg, G. Blomqvist, L. Ewald and K. Soff, *Ber.*, 69, 1258 (1936); K. H. Meyer, H. Hopff and H. Mark, *Ber.*, 62, 1103 (1929).

[30] K. Freudenberg and K. Friedrich, *Naturwissenschaften*, 18, 1114 (1930).

[31] M. Samec, *Kolloid-Beihefte*, 51, 378 (1940); K. H. Meyer, "*High Polymers*," 4, p. 387; Interscience Publishers, New York (1942). See also Chapter XII.

"amylopectin" of 207,000, for potato "amyloses" of 82,000 to 130,000, and for wheat "amyloses" of 90,000 to 156,000. Wheat starch purified by treatment with formamide at 120°C. is reported to have a molecular weight of 286,000 ($D.P.=$ 1770) in formamide solution. No significant change in the degree of polymerization is observed when the latter product is acetylated and subsequently deacetylated.[32] Extraction of starch grains under mild conditions gives an "amylose" fraction with molecular weight (after conversion to the acetate) of 10,000 to 60,000 whereas that for the "amylopectin" residue varies over the range 50,000 to 1,000,000.[33]

Application of the Ultracentrifuge. Studies carried out by use of the ultracentrifuge show that solutions of starch are extremely heterogeneous. Corn starch solubilized by dry grinding of the granules has been studied by Beckmann and Landis.[34] The "amylose" fraction remaining after electrophoretic separation of the "amylopectin" varies in molecular weight from 17,000 to 225,000 with nearly 50% of the material falling in the range 31,000 to 61,000. Determination of the form factors of fractions separated on the basis of their solubility in methanol-water mixtures indicates a great departure from spherical shape for the molecules composing the main fraction; only the particles composing the most soluble fraction have an approximately spherical shape.

Lamm has reported that the measurements are greatly influenced by the process used for dissolving the starch granule and employed concentrated solutions of zinc chloride or sodium mercury chloride to dissolve the material. The average particle size is of the magnitude of 4,000,000 under these conditions. Starches which have been treated with acids give diagrams with two peaks, which correspond to molecular weights of 60,000 and 200,000.[35]

Viscosity Measurements. The viscometric method is difficult to apply to the determination of the molecular weights of undegraded starches because of the difficulty of preparing starch solutions and because of the instability (retrogradation) of the solutions. A series of degraded starches, prepared by acid hydrolysis and purified by fractionation with methanol, has been used by Staudinger and Husemann for the determination of the K_m constant in the equation connecting viscosity and molecular weight:
$$\frac{\eta_{sp}}{c_{gm}} = K_m \cdot M.$$
The molecular weights determined osmometrically and the corresponding viscosities of formamide solutions of the products are used for the determination of K_m. An average value of 0.63×10^{-4} is reported

[32] H. Staudinger and E. Husemann, *Ber.*, *71*, 1057 (1938)
[33] K. H. Meyer, W. Brentano and P. Bernfeld, *Helv. Chim. Acta*, *23*, 845 (1940).
[34] C. O. Beckmann and Q. Landis, *J. Am. Chem. Soc.*, *61*, 1495 (1939)
[35] See: The Svedberg, *Ind. Eng. Chem., Anal. Ed.*, *10*, 113 (1938)

for K_m. The K_m values for the corresponding acetates and methylated derivatives also have been measured for several solvents.

A number of esters of unfractionated starch have been prepared by Mullen and Pacsu[36] under conditions involving minimal degradation. The molecular weights are calculated from the viscosities in pyridine solution using an approximate value of $K_m = 0.7 \times 10^{-4}$. The reported values for the triacetates of starches from different sources vary from 490,000 for rice starch triacetate to 510,000 for potato starch triacetate. These values correspond to 220,000 and 300,000 for the corresponding deacetylated starches.

The viscosity-concentration relationship for ethylenediamine solutions of amylose fractions is in agreement with the belief that they are linear polymers, but the molecular weights are probably somewhat greater[37] than the range 10,000 to 60,000 reported by Meyer.

Miscellaneous Methods. The reducing power of unmodified starches, although small, has been used to estimate the size of the starch molecule. Unmodified whole starches have reducing values between 2.8 and 8.9 mg. copper/g. starch. By use of this method molecular weights of the order of 74,500 to 238,000 (*D.P.* 460 to 1470) are obtained.[38]

The mercaptalation procedure of Wolfrom[39] leads to a degree of polymerization (*D.P.*) of about 150 glucose units for a sample of methylated potato starch which has a *D.P.* of about 7000 as measured by the viscometric method. Synthetic starch prepared by the action of potato phosphorylase on glucose phosphate (the Cori ester) exhibits a polymerization degree of 32 ± 1. The difference between the values obtained by the mercaptalation procedure and those obtained by the viscometric, end group and other methods remains unexplained.

F. End Group Assay and Molecular Structure. The quantity of tetramethylglucose obtained by the hydrolysis of methylated starches usually amounts to 3.8 to 4.6% of the theoretical.[40, 41] This quantity corresponds to one tetramethylglucose (one end group) for every 24 to 30 glucose units in the original material. If it is assumed that each molecule contains only one end group, the molecular weight must be of the order of 3000 to 5000. Such a conclusion is in direct contradiction to the values from osmometric,

[36] J. W. Mullen and E. Pacsu, *Ind. Eng. Chem.*, **34**, 1209 (1942).

[37] J. F. Foster and R. M. Hixon, *J. Am. Chem. Soc.*, **65**, 618 (1943); **66**, 557 (1944). See also: R. Speiser and R. T. Whittenberger, *J. Chem. Phys.*, **13**, 349 (1945).

[38] W. A. Richardson, R. S. Higginbotham and F. D. Farrow, *J. Textile Inst.*, **27**, 131T (1936).

[39] M. L. Wolfrom and D. R. Myers, *J. Am. Chem. Soc.*, **63**, 1336 (1941); M. L. Wolfrom, C. S. Smith and A. E. Brown, *ibid.*, **65**, 255 (1943).

[40] E. L. Hirst and G. T. Young, *J. Chem. Soc.*, 951, 1471 (1939)

[41] K. Hess and B. Krajnc, *Ber.*, **73**, 976 (1940).

ultracentrifugal and viscometric measurements, determined to be much greater than 100,000 and probably of a higher order of magnitude. The investigation of this anomaly has been carried out intensively by Haworth, Hirst, Hess, Staudinger and their associates. Although it was previously believed that the results might be interpreted on the basis of an aggregation of small molecules, the results are now explained by considering that some of the starch molecules consist of branched chains.

In order to reconcile the results obtained from the methylation studies with the observed molecular weights, Staudinger[42] has proposed a branched-chain structure for amylopectin which is represented schematically below. As noted above, each repeating unit has from 24 to 30 glucose residues which are distributed between the side chains and the main chain.

```
glucose                      glucose
  | 1                          | 1
  | 4'                         | 4'
(glucose)ₓ                   (glucose)ₓ
  | 1'                         | 1'
  | 6                          | 6
— -glucose -  (glucose)ᵧ   - glucose——  (glucose)ᵧ  — -
 —1        4 — 1'      4' — 1       4 — 1'       4'
  ‿_____   _____
```

Repeating units $(x + y = 22$ to $28)$
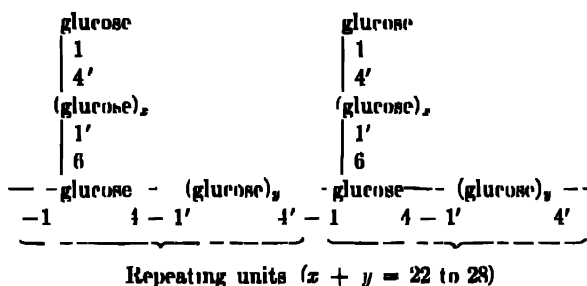
The terminal group of each chain, after complete hydrolysis of the methylated starch, should yield a molecule of tetramethylglucose. The glucose residue that is located at the other end of the side chain and that forms a part of both the main and the side chains should give a corresponding molecule of dimethylglucose. In actual practice, however, the amount of dimethylglucose in the hydrolysis products must be interpreted with considerable caution; if the methylation is not complete, the amount of dimethylglucose produced will be greatly affected. Hassid and McCready[43] report the following proportions of tetra-, tri- and dimethylglucoses in the products of the hydrolysis of methylated corn starch: 4 5, 90.7 and 4.7%, respectively. However, from starch methylated 12 to 15 times with dimethyl sulfate and alkali, Peat and Whetstone[44] isolated 4.6% "tetra", 84.5% "tri", and 10.9% "di".

The dimethylglucose fraction consists of 2,6- and 2,3-dimethylglucoses. The former in all probability is an artifact, for similar quantities are produced from methyl 2,3.6-trimethylglucoside under the same conditions as those used for the hydrolysis of the methylated starch. Since the corrected

[42] H. Staudinger and E. Husemann, *Ann.*, **527**, 195 (1937).

[43] W. Z. Hassid and R. M. McCready, *J. Am. Chem. Soc.*, **63**, 1632 (1941).

[44] S. Peat and J. Whetstone, *J. Chem. Soc*, 276 (1940).

amount of 2,3-dimethylglucose is equivalent to the quantity of 2,3,4,6-tetramethylglucose, there is one branching group for each end group. The isolation of the 2,3-dimethylglucose makes it probable that the side chains are connected to the main chains through 1,6' linkages.[15]

The preceding evidence shows that the starch molecule probably consists of a number of chains averaging 24 to 30 units in length, but it provides no evidence of the extent of deviation from this average length. It seems probable, however, that chains of 24 to 30 units may be fairly invariant structural units. This conclusion is supported by the constancy of the end group content of starches from all sources studied. Also, methylated starch can be hydrolyzed with oxalic acid in aqueous alcohol solution to give products with molecular weights as low as 20,000 (*D.P.* 120); all these degraded products contain the proportion of end groups present in the original material Hydrolysis to a greater extent, however, leads to products with a greater proportion of end groups. The bonds which are broken in the disaggregation process are primary valence bonds, for the rate of hydrolysis and the activation energy are comparable with those for the hydrolysis of simple glucosides [40][16] These results probably explain the apparently different effect of oxygen during the methylation of starch and of cellulose. The end group content of cellulose is greater when the methylation is carried out in air than when it takes place in nitrogen. Although disaggregation probably takes place for both materials, it results in an increase of end groups only for the straight cellulose chains

On the basis of the disaggregation experiments, it has been suggested that the starch molecule consists of parallel chains.[17] Each chain consists of 24 to 30 glucose units and is joined to a neighboring chain by a glucosidic linkage between its head group and the glucose unit of an adjoining chain, probably by 1,6' bonds. This laminated structure is illustrated schematically in the following diagram where the straight lines represent the parallel
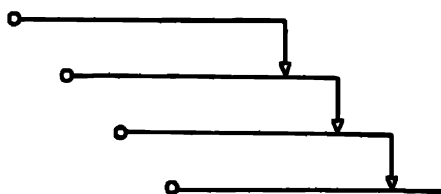
chains of 24 to 30 members; the arrows, glucosidic linkages between chains; and the circles, terminal end groups.

[15] K. Freudenberg and H. Boppel, *Ber*, **73**, 609 (1940); C. C. Barker, E. L. Hirst and G T. Young, *Nature*, **147**, 296 (1941).

[16] C E H. Bawn, E. L. Hirst and G T. Young, *Trans. Faraday Soc.*, **36**, 880 (1940)

[17] W N. Haworth, *Chemistry & Industry*, **17**, 917 (1939)

This structure differs from the branched structure proposed by Staudinger in that the laminated structure has no principal chain. Instead, it consists of a number of chains of 24 to 30 glucose units each; tne reducing group of each of these chains is connected by a glucosidic linkage (α-1,6?) to a second similar chain.
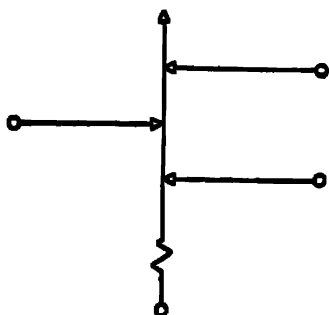
Branched Structure   Multiple Branched Structure
(Staudinger)   (Meyer)
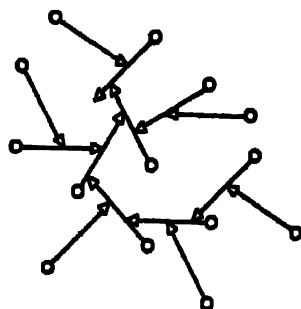
Meyer has proposed a multiple branched structure which has no central chain. This structure is supported by Myrbäck[18] as a result of considerations of the possible mechanism of synthesis by enzymes. The experimental evidence for these structures is meager, but it should be possible to obtain additional information. It would seem that the limit dextrin obtained by the action of β-amylase (see p. 587) should be a key substance for these studies.

As amylopectin has a molecular weight greater than 200,000 (D.P. > 1300) and has one end group for each 27 residues, the molecule must contain at least 50 branches. On the other hand, amylose is reported to have a molecular weight not greater than 50,000 (D.P. = 300) and to have an end group content of only 0.32% or one end group to every 300 residues. Inasmuch as the molecule can have only one terminal group according to these experiments, amylose must consist of a single straight chain of about 300 glucose units.[49] Hess and Steuer, however, find that their "amylose" has an appreciably larger molecule (D.P. 650 from osmometric determinations) and that three basic chains must be present.[50] Although the "amylose" fraction may contain some slightly ramified material it seems to be of much smaller molecular weight and to be significantly less branched

[18] See: K. Myrbäck, The Svedberg Mem. Vol., 508, (1944); Svensk Kem. Tid., 56, 60 (1944).

[49] K. H. Meyer, M. Wertheim and P. Bernfeld, Helv. Chim. Acta, 23, 865 (1940); 24, 378 (1941); W. Z. Hassid and R. M McCready, J Am Chem Soc., 65, 1157 (1943).

[50] K. Hess and E. Steurer, Ber., 73, 1076, 1317 (1940).

than amylopectin. Since there is considerable doubt whether the solubility methods used in these investigations are satisfactory for the separation of the amylose and amylopectin substances, it would be desirable to have these results checked by similar work on purer starch fractions.

G. X-Ray Diffraction Studies of Starch.[51] It has been shown by P. Scheirer and in more detail by Katz and his associates[52] that granular starches give X-ray diffraction patterns which altlough weak and diffuse indicate considerable crystalline organizatin. Usually, only powder diagrams have been obtained, and attempts at the preparation of oriented fiber diagrams have only recently been successful. Impurities have little effect on the patterns, for the patterns of crude and of purified starches exhibit little difference. The presence of moisture is important, however, and thoroughly dried starches produce only general scattering of the incident X-rays. The diagrams of granular starches usually fall into one of two groups and are classified as A or B diagrams. Some starches give patterns (C type) intermediate between the A and B types. As shown by Bear and French,[53] however, diagrams intermediate between the A and B types result from drying starch pastes at different temperatures; for products dried above 50°C, the diagrams are mainly of the A type; for products dried below 50°C., they rapidly approach the B type. From this evidence it seems probable that the C pattern represents states intermediate between those of the A and B modifications.

The ease of the transformation of the two types makes it probable that the corresponding lattices are not very different. An investigation of the powder diagrams of granular starches has been carried out by Bear and French who showed that all observed diffraction can be accounted for on the basis of the following parameters for the unit cells.

Modification	a_0	b_0	c_0	α	β	γ	Volume (A^3)
B	16.1	9.11	6 34	90.0	90.0	90.0	930
A	15.4	8 87	6 18	87.0	80.9	92 8	843

From the calculated volumes of the unit cells, the number of glucose residues in the unit cell is calculated as four when the density is taken as 1.50. These measurements were carried out on granular starches saturated with water. Under such conditions, the diagrams are much sharper than those obtained on drier products.

[51] M Samec and M. Blinc, *Kolloid-Beihefte, 52*, 57 (1940); D. French in "Chemistry and Industry of Starch", Editor· R. W. Kerr, p. 115; Academic Press, New York (1944).

[52] J. R. Katz and Associates, *Z physik. Chem., 184A*, 100 (1939), and many earlier articles in this Journal.

[53] R. S. Bear and D. French, *J. Am. Chem. Soc., 63*, 2298 (1941)

Film and fiber diffraction patterns of dried crystalline butanol-precipitated amylose have been obtained. The unit cell for the B-modification as calculated from these patterns is somewhat different from that obtained from the powder diagrams. It contains eight glucose residues and appears to be orthorhombic with the following dimensions:

$$a_0 = 16.0, \ b_0 = 10.6 \text{ and } c_0 = 9.2 \ A.$$

The B-modification of amylose probably consists of extended parallel chains of maltose residues. The general arrangement of the chains resembles that of cellulose and of chitin.[54]

Starches exhibit still another type of diagram called the V diagram.[51, 55] The V-modification is prepared by alcoholic precipitation from *freshly prepared* starch gels. It appears to have a helical structure; the amylose chains appear to be wound in a helix rather than to be extended linearly as in the B-modification.[54] Such a structure was originally proposed by Hanes[55a] to explain the action of enzymes on starch and to explain the colors produced by iodine on starch products degraded by enzymes. The amylose-iodine complex appears also to have the helical structure with the iodine atoms fixed along the axis of the helix and with one iodine atom for each turn.[56]

The complex formed between fatty acids and amylose resembles that of the amylose-iodine complex, and it appears to be formed of closely packed helical amylose spirals with fatty acids extended lengthwise along the axis of the spirals. Dipolar interactions rather than hydrogen bonds appear to be responsible for the bonding forces.[57]

Although amylopectin usually exhibits amorphous X-ray diagrams, it gives a crystalline pattern when allowed to retrograde under suitable conditions.[55] Granules of waxy maize starch, almost wholly consisting of amylopectin, yield an A-pattern.

3. Starch Esters

The three hydroxyl groups present in each glucose residue of the starch substances may be esterified, but the reaction proceeds with more difficulty than for cellulose, and considerable degradation usually takes place. Intact starch grains react with such difficulty that some of the earlier investigators concluded that no unsubstituted hydroxyl groups are present.

[54] R. E. Rundle, L. Daasch and D. French, *J. Am. Chem. Soc.*, **66**, 130 (1944).

[55] J. R. Katz, *Z. physik. Chem.*, *150A*, 37 (1930).

[55a] C. S. Hanes, *New Phytologist*, *36*, 101, 189 (1937).

[56] R. E. Rundle and D. French, *J. Am. Chem. Soc.*, *65*, 1707 (1943); R. E. Rundle, J. F. Foster and R. R. Baldwin, *ibid.*, *66*, 2116 (1944).

[57] F. F. Mikus, R. M. Hixon and R. E. Rundle, *J. Am. Chem. Soc.*, *66*, 1115 (1946).

Processes depending upon disruption of the granules at high temperatures or the use of acid catalysts lead to extensive degradation. Pyridine solutions of starch seem particularly valuable for obtaining esters since they may be prepared at temperatures of 90 to 100°C. and since the pyridine acts as a catalyst in the esterification reaction.[58]

Starch Acetates.[59] The various methods useful for acetylating the sugars may be applied to starch. As treatment of the intact granule at lower temperatures leads to products principally with the composition of a diacetate, it has been suggested that one of the hydroxyls of each glucose unit is protected from acetylation unless it is freed by a preliminary hydrolysis or acetolysis.[60]

Because the acetates frequently are utilized as intermediates in the preparation of methylated starches for end-group determinations, it is important that the acetylation process should produce no degradation. From the similarity of the reducing power of the original material and of the acetylated and deacetylated products, it appears that the pyridine method produces no molecular degradation. However, the use of chlorine and sulfur dioxide (Barnett catalyst) results in extensive degradation.[61] The molecular weights of starch acetates prepared by careful acetylation have been estimated by viscosity measurements to be approximately 400,000 to 550,000.[62] Some of the more degraded starch acetates, known by the English trade name of "Feculose," are said to be used for the manufacture of transparent sheets.

Films prepared from acetylated whole starch and amylopectin are quite brittle whereas those from the acetates of butanol-precipitated amylose (44.6 to 44.8% acetyl) are similar to those from high grade cellulose acetates. The amylose acetate films have greater inherent plasticity than the cellulose acetate films and require less plasticizer.[63]

Miscellaneous Organic Esters.[64] The benzoyl, tosyl and fatty acid esters of starch are prepared by the usual acylating methods (acyl chloride or the acid anhydrides in presence of pyridine or alkali). A monoformate is formed

[58] M. Bergmann and E. Knehe, *Ann.*, *458*, 141 (1927); H. Friese and F. A. Smith, *Ber.*, *61*, 1075 (1928); J. W. Mullen and E. Pacsu, *Ind. Eng. Chem.*, *34*, 1209 (1942); U. S. Patent 2,372,337, March 27, 1945; R. L. Whistler, *Advances in Carbohydrate Chem.*, *1*, 279 (1945).

[59] See: M. Samec, *Kolloid-Beihefte*, *51*, 369 (1940); L. Smith and R. H. Treadway, *Chem. Eng. News*, *22*, 813 (1944).

[60] W. S. Reich and A. F. Damansky, *Bull. soc. chim. biol.*, *19*, 158, 257 (1937).

[61] R. S. Higginbotham and W. A. Richardson, *J. Soc. Chem. Ind.*, *57*, 234 (1938).

[62] J. W. Mullen and E. Pacsu, *Ind. Eng. Chem.*, *34*, 1209 (1942).

[63] R. L. Whistler and G. E. Hilbert, *Ind. Eng. Chem.*, *36*, 796 (1944).

[64] For summary of methods used and esters prepared see J. W. Mullen and E. Pacsu, and R. L. Whistler, Ref. 58. For a summary of properties see: J. W. Mullen and E. Pacsu, *Ind. Eng. Chem.*, *35*, 381 (1943).

by the action of formic acid; from measurements of the amount of periodic acid consumed by the ester, the product appears to be substituted at the 6-positions. The ester gives a red color with iodine, but the regenerated starch, obtained by saponification of the monoformate, gives the original blue color.[65]

Starch Nitrates ("*Nitrostarches*"). Although starch nitrates have been known since the early part of the nineteenth century and although they are similar to the important cellulose nitrates, they have not achieved commercial importance. The nitration is carried out by reaction with nitric acid, usually in the presence of sulfuric or phosphoric acids,[66] or with N_2O_5.[67] By treatment with HNO_3-H_2SO_4 H_2O at 20°C. in the proportion 41: 56:3, products containing 11.0 to 13.3 % nitrogen are obtained. The X-ray diffraction pattern of starch nitrate appears in products of low degree of nitration.[68] The starch nitrates have received considerable attention for use as a military explosive and have been recommended[69] as demolition explosives to replace trinitrotoluene. Its resinous properties may lead to its application as a filler in lacquers.[70]

4. Ether Derivatives

Two types of starch ethers have received particular investigation, the methyl and benzyl ethers. The methyl ethers have played an important part in structural chemistry and are particularly important in the Haworth end group assay method. Their solubility in water and in organic solvents makes them of value for molecular weight estimations. The benzyl ethers,[71] which are made by action of benzyl chloride and sodium hydroxide, appear to be of some commercial interest as synthetic resins of the type particularly valuable for the production and treatment of electrical insulators.

A single treatment of starch by the dimethyl sulfate-alkali or methyl iodide-silver oxide method (p. 346) gives products with one-third to two-thirds of the hydroxyl groups methylated.[72] By repeated treatments, it is possible to obtain higher methoxyl contents, but the theoretical content (45.6% for $C_6H_7O_2(OCH_3)_3$) seldom is attained, although frequently twenty or more successive methylations are carried out. The number of treatments

[65] D. Gottlieb, C. G. Caldwell and R. M. Hixon, *J. Am. Chem. Soc.*, **62**, 3342 (1940).

[66] E. Berl and W. C. Kunze, *Ann.*, **520**, 270 (1935); J. Hackel and T. Urbanski, *Z. ges. Schiess. u. Sprengstoffw.*, **28**, 306, 350, 378 (1933); **29**, 14, 16 (1934)

[67] G. V. Caesar and M. Goldfrank, *J. Am. Chem. Soc.*, **68**, 372 (1946).

[68] G. Centola, *Gazz. chim. ital.*, **66**, 8 (1936).

[69] J. M. Young, *Military Engr.*, **31**, 11 (1939).

[70] A. Kraus, *Z. ges. Schiess. u. Sprengstoffw. Nitrocellulose*, **38**, 170 (1943).

[71] B. V. Maksorov and K. A. Andrianov, *Rev. gén. mat. plastiques*, **11**, 336, 373 (1935); M. Gomberg and C. C. Buchler, *J. Am. Chem. Soc.*, **45**, 1901 (1921).

[72] See: M. Samec, *Kolloid-Beihefte*, **51**, 369 (1940).

may be reduced greatly if a soluble starch derivative such as the acetate is used or if the reaction is carried out in the presence of chloroform.[73] Still fewer treatments are required if the acetylated starch in dimethylamine solution (or the partially methylated starch in anisole solution) is treated with sodium dissolved in liquid ammonia and finally with methyl iodide.[74] Products with a methoxyl content of 45.5% may be obtained in several treatments by the latter methods.

The ether derivative of starch and glycolic acid[75] has potential commercial interest because of the high viscosity and clarity of its aqueous solutions. It should resemble pectic acid in many ways because of the content of carboxyl groups. The carboxymethylstarch is made by the reaction of starch with chloroacetic acid.

$$
\underset{|}{\overset{|}{\text{HCOH}}} \;+\; \text{ClCH}_2\text{—COOH} \;\xrightarrow{\text{NaOH}}\; \underset{|}{\overset{|}{\text{HCOCH}_2\text{—COOH}}}
$$
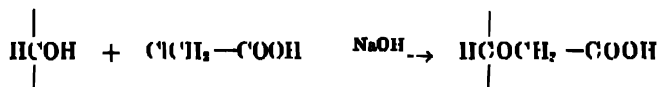
Carboxyethyl esters of starch, soluble in water,[76] can be made by treatment of alkali starch with acrylonitrile.

Allyl starch[77] is prepared by the action of allyl bromide or chloride on starch or acetylated starch in the presence of alkali. The product polymerizes in the presence of air or peroxides to give a resinous product that may have value as a component of lacquers and other coatings.

5. Oxidation Products[78]

Products obtained by the action of oxidizing agents on raw starches are of considerable industrial importance, particularly as sizing materials. In general, the chemistry of the reactions and of the products are unknown since most of the theoretical studies have involved much more intensive oxidation than takes place in the commercial processes. Because of the increased fluidity of dispersions of oxidized starches as compared with untreated starches, they are classified with acid-treated ("acid-modified") starches as "thin-boiling starches." In addition they give clearer solutions

[73] W. N. Haworth, E. L. Hirst and J. I Webb, *J. Chem Soc.*, 2681 (1928); W. Z. Hassid and W. H. Dore, *J. Am. Chem. Soc.*, **59**, 1503 (1937).

[74] K. Freudenberg and W. Rapp, *Ber.*, **69**, 2041 (1936), K. Hess, H. A. Schulze and B. Krajnc, *ibid.*, **73**, 1069 (1940).

[75] J. K. Chowdhury, *Biochem. Z.*, **148**, 76 (1924); F. Hoppler, *Chem. Ztg.*, **17**, 72 (1943).

[76] British Patents 546,585 of July 1, 1943 and April 10, 1944; 564,585, Oct. 4, 1944.

[77] C. G. Tomecko and R. Adams, *J. Am Chem. Soc.*, **45**, 2698 (1923); P. L. Nichols, R. M. Hamilton, L. T. Smith and E. Yanovsky, *Ind. Eng Chem*, **37**, 201 (1945).

[78] J. M. Newton and O. T. Peckham, Jr., *in* "Chemistry and Industry of Starch," R. W. Kerr, Editor, p. 224; Academic Press, New York (1944)

that have a lower congealing rate and that are more adhesive than those of unmodified starches. These commercial products, however, still retain granular structures, are difficultly soluble in cold water, and give the usual colors in the presence of iodine.

Two general types of oxidation are used for the commercial processes: hypochlorite (and halogen) and peroxide. The first type is carried out by treating a slightly alkaline starch slurry with sodium or calcium hypochlorite and allowing the reaction to take place at 32-52°C. When the desired degree of oxidation is reached, sodium bisulfite is added to neutralize the oxidizing agent, and the product is ready for use after the water has been removed. Oxidative modification of starch also may be brought about by the action of alkaline peroxides, permanganates, persulfates and perborates; the reactions may take place in the wet or dry states, but elevated temperatures are necessary.

Although the course of the oxidizing action of hypochlorites and halogens in the preparation of commercial oxidized starches is largely unknown, there is no doubt that it is quite complex. Alkaline hypoiodites oxidize free aldehyde groups to carboxyl groups. This reagent has been used for the determination of free aldehyde groups in starches and consequently of chain lengths.[79] However, bromine oxidation of starches and starch derivatives apparently takes place through oxidation of the primary hydroxyl groups to carboxyl groups and the formation of glucuronic acid residues.[80, 81] Bromine oxidation also may lead to a cleavage of the carbon chains as is evidenced by the separation of dibasic acids with carbon chains shorter than six atoms in length. The bromine-oxidized starch also forms an oxime derivative.[81] This reaction has been attributed to the formation of a ketone group during the oxidation process.

In contrast to the action of halogens, periodic acid is extremely specific in its action. As noted in an earlier chapter, periodic acid oxidizes glycol groups to aldehyde groups with simultaneous cleavage of carbon-carbon linkages. Starch, treated with periodic acid, retains its granular appearance but loses its birefringent properties. The reaction proceeds fairly rapidly until a molecule of the oxidant is utilized per glucose residue, and thereafter it progresses quite slowly. The product obtained is soluble in hot water, does not give a color with iodine and is not attacked by amylases.[82]

The periodate ion is able to penetrate the starch granules, and powdered whole starch and soluble starch react at about the same rate.[83] Except for

[79] M. Levine, J. F. Foster and R. M Hixon, *J. Am. Chem Soc.*, **64**, 2331 (1942).

[80] V. Syniewski, *Ann.*, **441**, 277 (1925)

[81] F. F. Farley and R M. Hixon, *Ind Eng. Chem*, **34**, 677 (1942).

[82] E L Jackson and C S Hudson, *J. Am Chem. Soc*, **59**, 2049 (1937); **60**, 989 (1938).

[83] D. H. Grangaard, E. K. Gladding and C. B Purves, *Paper Trade J.*, **115**, No. 7, 41 (1942).

the reducing end residues, the glucose units in the starch chains should be oxidized to dialdehydes which on hydrolysis should yield glyoxal and D-erythrose. The reducing end residues, however, should each yield three molecules of formic acid and one of formaldehyde. Hence, the determination of the formaldehyde produced in the reaction provides a means for the determination of the end group content.[51]

Single glucose residue of   Single unit
starch chain   of oxidized
                                   starch
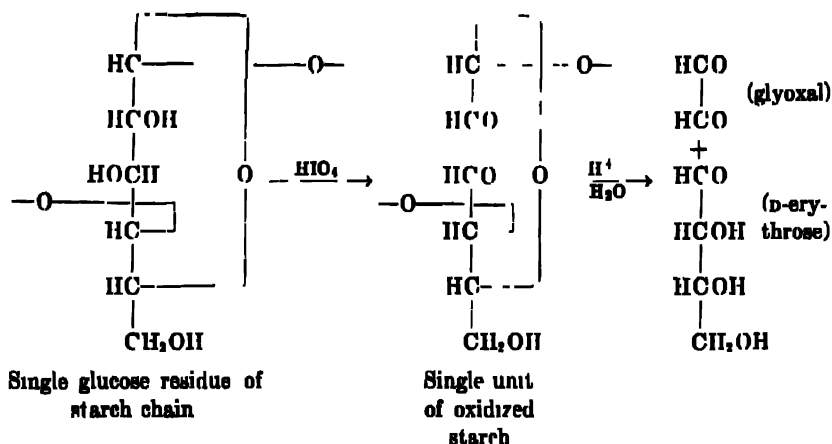
As shown above, glyoxal and D-erythrose should be the main products in the hydrolyzates of starches oxidized with periodic acid. The glyoxal is isolated readily from the hydrolyzates after conversion to the osazone.[51] Conditions have been devised whereby 90% recovery of the glyoxal units[52] is attained. The glyoxal interferes with the identification of the D-erythrose, but after its removal by oxidation with bromine to oxalic acid and precipitation with barium, the D-erythronic acid may be identified as its crystalline brucine salt and lactone.[52] The isolation of these products provides evidence that the course of the reaction is similar to that for the simpler glycosides and that the principal feature of the starch structure is a series of glucose residues attached through 1,4′ glucosidic bonds.

6. The Action of Enzymes on Starch[53]

The degradation of starch by enzymes has great industrial and biological significance because it is involved in the preparation of most alcoholic cereal beverages (beer and whiskey), in bread making and in the preparation of certain textile sizes, adhesives, etc. Its biological importance arises

[51] C. G. Caldwell and R. M. Hixon, *J. Biol. Chem.*, *123*, 595 (1938).

[53] C. S. Hanes, *New Phytologist*, *36*, 101, 189 (1937); M. Samec and M. Blinc, *Kolloid-Beihefte*, *49*, 75 (1939); K Myrback, *J. prakt. Chem*, [2] *163*, 29 (1943); J.A. Anderson (Editor), "Enzymes and Their Role in Wheat Technology;" Interscience Publishers, New York (1946)

from its function in supplying carbohydrate for the metabolic needs of animals. In spite of extensive investigations, the subject is in a state of confusion, and many conflicting claims are to be found. In the present discussion it will be possible to discuss only a small fraction of the work which has been carried out.

One of the most important industrial sources of amylases is barley malt, which is prepared by allowing barley to germinate. Bourquelot and Märcker[66] postulated the existence of at least two different amylases in barley malt. The classical experiments of Wijsman demonstrate their existence particularly well. Following Wijsman's method,[67] the presence of the two amylases is demonstrated by placing a drop of malt extract on a starch-gelatin plate and allowing the plate to remain in an ice box for several days. When the plate is stained with iodine most of the surface immediately turns blue-black indicating unchanged starch, but where the droplet has been placed, a colorless disc surrounded by a mauve annular ring is formed. Sections of the mauve ring may be cut out and transplanted to fresh starch-gelatin plates, which after several days are stained as before. In this case only a mauve disc without a colorless center is seen on the blue-black background. These results are explained by assuming that two enzymes with different rates of diffusion are present in the malt extract. The component with the greatest diffusion rate degrades starch to a substance giving a mauve color with iodine whereas the more slowly diffusible enzyme carries the hydrolysis to substances giving no color with iodine.

Most workers agree that at least two main types of amylases exist and that the two found in malt are fairly typical of those from other sources. The one type produces a rapid decrease in the viscosity of starch gels with the formation of reducing dextrins and sugars and with the destruction of the components giving colors with iodine. The second type, however, has but little effect on the viscosity of starch gels, forms much reducing sugar (mainly maltose) and forms other products from whole starches that are colored blue by iodine. Since the action of members of the first group is characterized by a rapid decrease in the viscosity of the solution, they are called liquefying-amylases (also dextrogenic or α-amylases); the members of the second group are named saccharifying or β-amylases. In analogy to the mutarotation of α- and β-maltose, the names alpha and beta originally were given to indicate that the one (alpha) gives products mutarotating downward and the other (beta) substances mutarotating upward.[68]

[66] E. Bourquelot, *Compt. rend*, *104*, 576 (1887), Märcker, *Landw Vers.-Sta.*, *23*, 69 (1879).

[67] H. P. Wijsman, as quoted by C S Hanes, *loc. cit.*, p 137

[68] R. Kuhn, *Ann.*, *443*, 1 (1925); G G Freeman and R. H. Hopkins, *Biochem. J.*, *30*, 451 (1936)

Amylases attack intact starch granules only with difficulty. Earlier investigators have attributed this resistance to hydrolysis as arising from the presence of a protective material in the surface of the granule. A more probable explanation is that the rate is slow because the reaction is heterogeneous in nature and that the granules are not penetrated by the enzyme. Fairly high degrees of enzymic hydrolysis of raw starches have been observed when a mixture of pancreatic and fungal amylases are allowed to act on starch granules at temperatures below the gelatinization point.[89]

A tentative classification[90] of the amylases follows:

Classification of Amylases

Class	Examples
Saccharifying amylases	β-Amylases of wheat, barley, soybeans, etc
Liquefying amylases (Dextrogenic ")	
Group 1	Malt and other cereal amylases; *Aspergillus* amylases.
Group 2	Pancreatic, *B mesentericus*, salivary amylases.
Group 3	*B. macerans* amylase
Phosphoamylases	(Operating by phosphorylation process.)
1. Phosphorylases of Hanes and Cori	Potato, muscle phosphorylases
2. Disaggregating amylase of Waldschmidt-Leitz and Mayer	(Existence questionable)

As in malt, the saccharifying and liquefying amylases often occur together. A study of the comparative liquefying and saccharifying power of numerous seeds and vegetables shows that the liquefying enzymes are very common, but the saccharogenic enzymes were found only in rye, barley, wheat, soybeans and potatoes. When barley germinates to form malt, both activities increase, but the liquefying ability increases much more than the saccharifying ability.[91] Sodium chloride and other salts frequently increase the activity of these enzymes, particularly the liquefying amylases.

As shown in Fig. 3, the malt amylases are influenced to different degrees by changes of acidity. Those from other cereals exhibit similar differences. The liquefying amylases of type 1 (see Classification) are most active at pH 4 to 6; these of class 2 operate most efficiently near the neutral point (pH 6 to 8).

[89] A. K. Balls and S. Schwimmer, *J. Biol. Chem*, **156**, 203 (1944).

[90] W. W. Pigman, *J. Research Natl. Bur. Standards*, **33**, 105 (1944).

[91] H. C. Gore and S. Józsa, *Ind. Eng. Chem*, **24**, 102 (1932); T. Stenslam, C. O. Björling and E. Ohlsson, *Z. physiol. Chem.*, **226**, 265 (1934)

The dormant grains usually are much richer in β-amylases than α-amylases. The germinated or "malted" grains provide richer sources of the α-amylases. Barley, wheat and rye are representative of this behavior. Ungerminated maize, millet, sorghum and rice, however, appear to be practically devoid of α- and β-amylase activity.[92] Malts prepared from sorghum, maize and oats are said to contain only α-amylases; soybeans and ungerminated barley, wheat, rye and oats contain only the β-amylase component. The amylase of the white sweet potato is reported by Shukla[93] to be of the β-type. Those present in the tubers of a number of East Indian
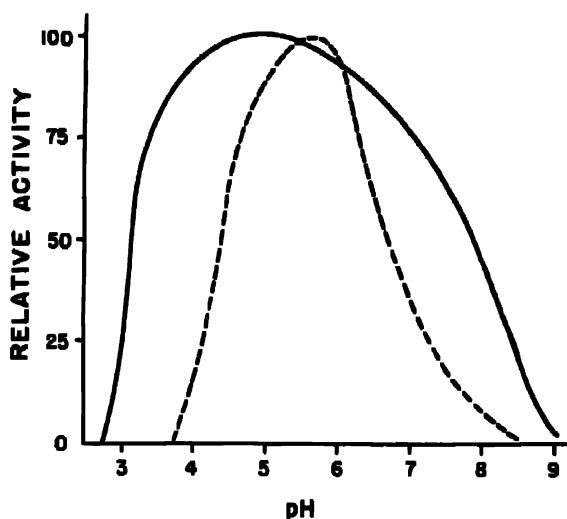
Fig. 3. The influence of pH on the activity of the malt enzymes
The full line gives data for the saccharogenic β-amylase and the dotted line that for the liquefying α amylase (after E Ohlsson).

plants used widely as foods have also been studied. Those of arvi (*Colocasia antiquorum*) and banda (*Zingiber cassumunar*) tubers may be of the α-type since their optimal pH is similar to that of malt α-amylase.

As the individual amylases exhibit considerable differences in properties such as in the stability to destructive influences, enzyme mixtures like malt may be separated at least partially. Schwarzer (1870) and later Kjeldahl and O'Sullivan showed that treatment of malt at temperatures above 60°C. affects the liquefying (or dextrinizing) action of the malt less

[92] E. Kneen, *Cereal Chem.*, *21*, 304 (1944).
[93] J. P. Shukla, *J. Indian Chem. Soc.*, *21*, 223 (1944) and earlier articles.

than the saccharifying action. Methods were described later by which either of the two components may be selectively inactivated.[14]

Although malt α-amylase is resistant to a short heat treatment at 70°C, it is rapidly inactivated by acids at room temperature. In contrast, the malt β-amylase is resistant to acids and is destroyed rapidly at 70°C. Malt α- and β-amylases, made by preferential inactivation of the other component, have been used extensively but are of questionable freedom from the second component. A better separation of the two enzymes results from the utilization of the stability of malt and wheat α-amylases and the instability of the corresponding β-amylases to calcium ions. At a pH of 6.0 to 7.0, at a temperature of 70°C. and in the presence of calcium ions, the β-amylases are completely inactivated whereas the α-amylases are scarcely affected. Conversely, at a pH of 3.0, at a temperature of 30°C. and in the absence of calcium ions, the α-amylases of wheat and barley are destroyed although the β-amylase activity is only partially impaired.[15]

The study of the action of amylases on starches has been beset with many difficulties in addition to those common to most investigations in the starch field. In general, degrees of hydrolysis have been determined by reducing sugar measurements and calculated as maltose. However, reducing values may be meaningless as a quantitative measure since the products of hydrolysis include dextrose and reducing dextrins (oligosaccharides) in addition to maltose. In some instances, degrees of hydrolysis greater than 100 have been observed because of the presence of dextrose in the hydrolysis products.[16] Hence, the measurement of fermentable sugars present in the products often provides a more significant quantitative measure of hydrolysis than reducing sugar determinations.[20] Selective determination of glucose, maltose and dextrins provides a still more significant but more tedious procedure.[16]

The questionable purity of the amylase preparations in use also makes it difficult to ascribe definite actions to individual enzyme components. Thus, malt and most cereal emulsins contain the α- and β-amylases. Many fungal, pancreatic and bacterial emulsins have highly active liquefying enzymes; they also contain enzymes which synthesize oligosaccharides from maltose.[20] The synthesizing action of most amylase preparations usually has not been considered and may be the explanation for many discrepancies.

A. Saccharogenic or β-Amylases. The β-amylases, which are found in many dormant plant seeds, have been obtained in a highly active condition

[14] E. Ohlsson, *Z physiol. Chem.*, *189*, 17 (1930); J. Blom, A. Bak and B. Braae, *ibid.*, *250*, 104 (1937).

[15] E. Kneen, R. M. Sandstedt and C. M. Hollenbeck, *Cereal Chem.*, *20*, 399 (1943); W. J. Olson, B. A. Burkhart and A. D. Dickson, *ibid.*, *20*, 126 (1943)

[16] See· I E. Stark, *J Biol. Chem.*, *142*, 569 (1942).

from malt and soybeans.[97] Wheat, soybeans and sweet potatoes supply fairly pure sources of β-amylases,[98] and from sweet potatoes a crystalline enzyme of this type has been obtained.[99]

The freedom of β-amylase preparations from liquefying amylases may be tested by studying their action on the limit dextrin (see below).[100]

A method of evaluating the activity of β-amylases, which may be used in the presence of α-amylases, is described by Kneen and Sandstedt.[100, 101]

When the enzymes act on solutions of whole starches, the hydrolysis proceeds rapidly until about 50 to 55% of the theoretical quantity of maltose is produced and then very slowly until a limit of about 60 to 67% is reached.[99, 102] With β-amylases, the sole hydrolysis products usually are maltose and a non-reducing dextrin. The molecular weight of the dextrin (determined osmometrically) is about 79,000 ($D.P.$ about 400). Methylation data show that there is one end group for each 11 to 12 glucose residues. Hence, the number of repeating units in the dextrin is about 36. Many names for this dextrin are found in the literature; some names are: erythrogranulose, α-amylodextrin, dextrin-A, grenzdextrin and residual dextrin. The dextrin is colored blue by iodine. It resists further attack by β-amylases unless treated by other enzymes or by autoclaving.[103] Earlier reports of the slow hydrolysis of the residual dextrin by β-amylase preparations probably are to be ascribed to the presence of liquefying amylases in the β-amylase preparation.

The amylose fraction of starch is hydrolyzed to maltose practically quantitatively by β-amylases.[104] Reports of incomplete hydrolysis probably are due to the presence of amylopectin or of insoluble material in the amylose.

The high yields of maltose obtained by the action of β-amylases on amylose, which has a straight-chain structure, suggest that these enzymes act by a continuous process of cleavage of the maltose groups from the ends of the chains.

[97] H. C. Sherman, M. L. Caldwell and S. E. Doebbeling, *J. Biol. Chem.*, *104*, 501 (1934); O. Holmbergh, *Srensk Kem. Tid*, *50*, 253 (1933).

[98] J. M. Newton, R. M. Hixon and N. M. Naylor, *Cereal Chem.*, *20*, 23 (1943); K. V. Giri, *J. Indian Chem. Soc.*, *15*. 219 (1938)

[99] A. K. Balls, R. R. Thompson and M. K. Walden, *J. Biol. Chem.*, *163*, 571 (1946)

[100] W. J. Olson, R. Evans and A. D Dickson, *Cereal Chem.*, *81*, 533 (1944).

[101] E. Kneen and R. M. Sandstedt, *Cereal Chem.*, *18*, 237 (1941).

[102] For example see· J. Blom, A. Bak and B. Braae, *Z. physiol. Chem.*, *241*, 273 (1936).

[103] W. N. Haworth, H. Kitchen and S Peat, *J. Chem. Soc.*, 619 (1943).

[104] A. R. Ling and D. R. Nanji, *J. Chem. Soc.*, *127*, 629 (1925); M. Samec, *Z. physiol. Chem.*, *236*, 103 (1935); K. H Meyer, W. Brentano and P. Bernfeld, *Helv. Chim. Acta*, *23*, 845 (1940).

The amylopectin fraction of starch is hydrolyzed only to the extent of 54 to 60% (calculated as maltose) and with the production of the non-reducing residual dextrin.[105] The results with the two starch fractions are in disagreement with the early concept that whole starch consists of two sub-stances, one (α-starch) hydrolyzed by α-amylases and one (β-starch) acted on by β-amylases.

In the case of amylopectin, it is believed that the hydrolytic action of the enzyme also proceeds from the ends of the chains until a point of branching occurs or until some unusual type of linkage is reached. Hence, the residual dextrin should exhibit an unusually high proportion of end groups. In agreement with such a mechanism, it is reported that the residual dextrin contains one end group for every 10 to 12 glucose residues[106] as compared with one for every 25 to 30 residues for the original starch.

Straight chain fragments from starch with the free aldehyde groups oxidized to carboxyl groups are also hydrolyzed by β-amylases. Hence, it appears that the enzyme attacks the nonreducing end of the molecule and proceeds along the chain. Maltohexonic acid (an oxidized hexasaccharide) yields as a result of enzymic hydrolysis two molecules of maltose and one of maltobionic acid. The minimal chain length necessary for β-amylase action appears to be four (two maltose units) since maltotriose (a tri-saccharide) is not hydrolyzed.[107]

After long action of β-amylases on residual dextrins (which action may be due to the presence of other amylases in the enzyme preparation) a disac-charide is obtained which appears to be mainly isomaltose, 6-glucose α-glucoside. The isomaltose type of linkage is hydrolyzed by acids at a much slower rate than the maltose type.[108] It is also claimed that products con-taining alpha 1,3′ linkages are obtained.[109]

Highly purified β-amylase preparations from barley and barley malt have been studied[110] from the viewpoint of their chemical composition. The materials are protein in character. The activity of a number of prep-arations was found to be approximately proportional to the number of sulfhydryl groups as determined by the nitroprusside reaction. Iodine inactivates the β-amylase, but about 10 to 15% of the activity is recovered after treatment with hydrogen sulfide or cysteine. It is probable that the irreversible loss of activity is a result of the iodination of tyrosine to form

[105] G. Freeman and R. H. Hopkins, *Biochem. J.*, **30**, 446 (1936); K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **23**, 875 (1940).

[106] K. H. Meyer, M. Wertheim and P. Bernfeld, *Helv. Chim. Acta*, **24**, 212 (1941).

[107] K. Myrbäck and G. Nyrander, *Biochem. Z.*, **311**, 234 (1942).

[108] K. Ahlborg and K. Myrbäck, *Biochem. Z.*, **308**, 187 (1941).

[109] Y. Nakamura, *Bull. Agr. Chem. Soc., Japan*, **17**, 77, 603 (1941).

[110] C. E. Weill and M. L. Caldwell, *J. Am. Chem. Soc.*, **67**, 212, 214 (1945).

diodotyrosine. The reversible loss of activity presumably arises from oxidation of sulfhydryl groups to disulfide linkages; confirmation for this conclusion is obtained by the inactivation resulting from the action of reagents specific for SII groups: aryl-mercuric compounds, nitroprusside reagent and iodoacetamide. On the other hand, free amino groups apparently are not essential for the action of the barley β-amylase.

B. Liquefying Amylases. Enzyme preparations (emulsins) made from bacteria, fungi, pancreases, saliva and cereals contain amylases which rapidly liquefy starch gels. Because there is a rapid production of low molecular weight dextrins, these enzymes are known as dextrinogenic amylases as well as liquefying amylases. As will be shown later, both activites appear to be due to the same enzymes.

Many workers use the term α-amylase synonomously with liquefying amylases. However, as there is some evidence that the cereal α-amylases may act differently from the bacterial, pancreatic and salivary enzymes, the term α-amylases will be limited to the liquefying cereal amylases in the present discussion. Since many of the enzyme preparations are able to synthesize oligosaccharides from maltose,[90] it is difficult to compare the actions of these enzymes.

The liquefying amylases are of first importance in most of the industrial processes employing amylases. Unfortunately, this fact has not been realized generally, and the customary method of measuring amylase activity evaluates mainly the β-amylase activity. Thus, the Lintner method in general use depends upon measuring the reducing sugar formed during a brief action of the enzymes on a soluble starch solution.

Although the cereal α-amylases and *Aspergillus* amylases may bring about high degrees of saccharification, sugar formation takes place mainly in the latter stages of the reaction. Malt α-amylase hydrolyzes starches to fermentable reducing sugars in about 90% of the theoretical yield.[90, 111] In the presence of yeasts, the cereal α-amylases and the *Aspergillus* enzymes break down starches completely to fermentable sugars. The other liquefying amylases and the β-amylases can bring about only partial hydrolysis of starches to fermentable sugars in the presence of yeasts.[90]

Either the liquefying power or the dextrinogenic action may be used as a measure of the action of these enzymes, but it is not well to compare enzymes from different sources. The liquefying power is determined[112] by viscometric measurements on a standard potato starch gel during the period of hydrolysis. During the liquefaction period, the products of hydrolysis rapidly lose their power to give colored products with iodine. The time

[111] R. H. Hopkins and D. Kulka, *Wallerstein Comm.*, **5**, 115 (1942).

[112] S. Józsa and W. R. Johnston, *Ind. Eng. Chem., Anal. Ed.*, **7**, 143 (1935); J. Blom and A. Bak, *Z. physiol. Chem.*, **256**, 197 (1938).

required for the starch to be degraded to products which produce no color with iodine may be used for the measurement of the enzyme activity.[113] Since β-amylases may exert a slight influence on the rate of dextrinization, it is suggested that the determination be carried out in the presence of an added excess of β-amylase.

Hollenbeck and Blish[114] compared the liquefying power and the dextrinizing power of malt, *A. oryzae* (Takadiastase) and bacterial amylases and showed that the two actions are probably produced by the same enzymes. Both types of activity are affected in like degree by heat inactivation, and the optimal pH for both actions is the same although it varies with the enzyme source. However, the ratio of dextrinizing to liquefying power varies for enzymes from different sources.[115]

The changes in the color of the starch-iodine complex have been measured, with the aid of a spectrophotometer, at various stages of the hydrolysis of starch by liquefying amylases.[116] As the hydrolysis progresses, the color changes rapidly from blue through violet to red-brown, and finally there is a slow transformation through orange to deep yellow. The absorption maximum, initially around 5700 $\mathring{A}$, shifts to the ultraviolet as the reaction proceeds. This behavior is in contrast to the action of β-amylases in which case there is no shift in the absorption maximum.

The stage of degradation at which the hydrolysis products do not change the color of iodine solutions is designated as the "achroic point" and the reducing value (calculated as maltose) at this point has been termed the *achroic R value*. For malt α-amylase, the achroic point is reached at 28 to 31% conversion to maltose. Other liquefying amylases produce more reducing sugar before the achroic point is reached. For *Aspergillus* amylases the *R value* is 44%; for salivary amylase, 46%; and for pancreatic amylase, 55%.

The nature of the products obtained by the action of liquefying amylases on starch has been investigated,[117] but the problem is complicated by the questionable freedom of the enzyme preparations from β-amylase, maltase (α-glucosidase) and other enzymes. Various methods such as alcoholic precipitation and the selective fermentation of maltose and dextrose have

[113] J. Wohlgemuth, *Biochem. Z.*, **9**, 1 (1908); R. M. Sandstedt, E. Kneen and M J Blish, *Cereal Chem.*, **16**, 712 (1930); W. J. Olson, R. Evans and A. D. Dickson, *ibid.*, **21**, 533 (1944).

[114] C. M. Hollenbeck and M. J. Blish, *Cereal Chem.*, **18**, 754 (1941); J. Blom, A. Bak and B Brane, *Z. physiol. Chem.*, **250**, 104 (1937).

[115] S. Redfern and Q. Landis, *Cereal Chem.*, **23**, 1 (1946).

[116] C S. Hanes and M. Cattle, *Proc. Roy. Soc.* (*London*), **B125**, 387 (1938).

[117] See reviews by M Samec and M. Blinc, *Kolloid-Beihefte*, **49**, 117 (1939), by K Myrback, *J. prakt Chem.*, [2] **163**, 29 (1943) and by C. S. Hanes, *New Phytologist*, **36**, 101, 189 (1937).

been used to determine the nature of the products. At the achroic point (28-31% hydrolysis to maltose for malt α-amylase), the hydrolytic product consists of dextrins. According to Hanes these dextrins are composed of 6 to 7 glucose units each. In the later stages of hydrolysis, considerable maltose is formed.

The dextrins produced by the action of salivary amylase, malt α-amylase and *Aspergillus oryzae* amylase (Takadiastase) have been studied by K. Myrbäck and associates.[11b] From the products of action of salivary amylase, about 25% of non-fermentable dextrins are obtained. Of these about one-quarter consists of dextrins with between 6 and 8 glucose residues, about one-half of dextrins with 6 residues and one-quarter of dextrins with between 4 and 6 residues. Long action of malt α-amylase on rice starch yields some hexasaccharides but mostly tetra- and trisaccharides as the components of the unfermentable fraction. *A. oryzae* emulsin produces more hexasaccharides (about two-thirds of the nonfermentable material) and some tri- and tetrasaccharides. Wheat, barley and potato starches appear to give residual dextrins which are much more heterogeneous than those from corn, arrow-root and rice starches. Rarely, however, are residual dextrins with more than 12 glucose units found when hydrolysis is allowed to reach completion.

If hydrolysis by α-amylases proceeds from the ends of chains, it would be expected that an interruption of the hydrolysis would leave a portion of the original starch molecule which should be of high molecular weight. However, when malt α-amylase is allowed to act only very briefly on starch so that only 10% hydrolysis is attained (from reducing values), the largest dextrins are composed of about 23 glucose residues and at 18.1% hydrolysis the largest dextrins have a degree of polymerization of only 13. Since there are no high molecular weight dextrins as is the case for β-amylase action, the hydrolysis probably does not proceed from the end of the chains. At a hydrolysis degree of 21.6%, the non-fermentable dextrin fraction consists of two-thirds of hexa- and heptasaccharides and one-third of tetra- and pentasaccharides.

The dextrins produced at the lower degrees of hydrolysis (10 to 20%) differ in their behavior to β-amylase. Although those composed of less than about seven glucose residues are completely broken down by this second treatment to fermentable sugars (maltose and maltotriose, those with longer chains are only partially hydrolyzed by β-amylase to fermentable sugars. According to Myrbäck, this resistance of the longer-chain dextrins to hydrolysis is due to the presence of isomaltose (1,6') linkages which block the action of β-amylase. The existence of abnormal linkages is also

[11b] K. Myrbäck, *J. prakt. Chem.*, [2] 152, 29 (1943).

indicated by the resistance of these same dextrins to acid hydrolysis. Since the dextrins obtained by the action of malt α-amylase on potato starch exhibit a correlation between the molecular weight and the phosphorus content, it is possible that esterified phosphate groups may play the same rôle as 1,6′ linkages in stopping the action of β-amylase.

In accordance with the suggestion of Hanes, the action of liquefying amylases is believed to consist of a hydrolysis of starch chains into fragments mainly comprised of hexasaccharides. This mechanism receives support by the preparation of dextrins from straight chain "amyloses" by the action of liquefying amylases.[118, 119] The unfermentable dextrins present when the hydrolysis has proceeded to an extent of 21% (calculated as maltose) consist mainly of hexa- and heptasaccharides (65%) and tetra- and pentasaccharides (32%).

The dextrinization by malt α-amylase does not appear to take place by the action of the enzyme on the end of chains, but as mentioned above, the absence of unhydrolyzed fragments of high molecular weights after short periods of hydrolysis indicates that the enzyme action may take place in central portions of the molecule. The presence of 1,6′ (isomaltose or, better, isogentiobiose) linkages in the dextrins shows that the enzyme action may take place over these linkages which are able, however, to block the action of β-amylase. Hydrolysis of normal hexasaccharides to maltose is brought about by both α- and β-amylase preparations, but the reaction may be caused by the presence of dextrinases or α-glucosidases (maltases).

Efforts to separate malt α-amylase from a dextrinase by adsorption methods have not succeeded, and at present there is no evidence for such an enzyme.[120]

The presence of dextrins of about 24 units in length among the products of a brief action of liquefying amylases on starch as noted by Myrbäck (see above) suggests that the primary action of these enzymes involves the disruption of the branched chain component into its basic short chains of 24 to 30 glucose units in length.

Studies[121] of the inactivation of purified pancreatic amylase by reagents specific for certain free groups show a marked difference in the nature of active groups as compared with barley β-amylase (see above). The amino groups of the pancreatic enzyme are essential to its activity whereas the sulfhydryl groups are not.

C. Schardinger Dextrins.[122] The cultivation of *Aerobacillus macerans* (*Bacillus macerans*) upon starch solutions leads to the production of water-

[118] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **24**, 350E (1941).

[120] K. Myrbäck and Per J. Palmerantz, *Arkiv Kemi Mineral. Geol.*, *18A*, No. 6 (1944); *Chem. Abst.*, **39**, 3799 (1945).

[121] M. L. Caldwell, C. E. Weill and R. S. Weill, *J. Am. Chem. Soc.*, **67**, 1079 (1945).

[122] M. Samec and M. Blinc, *Kolloid-Beihefte*, *49*, 211 (1939).

soluble dextrins (called Schardinger dextrins) of which two have been obtained in crystalline form.[123] Bacteria-free filtrates of cultures of *Aerobacillus macerans* contain the enzyme responsible for the formation of the Schardinger dextrins and produce from starch 60% yields of the dextr; is.[124] In the initial stages of the reaction, the more soluble α-dextrin is formed in maximum yields of 20%, but after many days the yield of the β-dextrin reaches 22%, and the α-dextrin practically disappears. During the initial stages, a rapid decrease in the viscosity of the solution takes place. Almost no reducing sugars are formed in the reaction. The dextrins are characterized by their behavior in the presence of iodine; under the microscope, the α-dextrin iodine compound appears as green needles or blue hexagons whereas the β-dextrin iodine compound shows up as brown prisms.

Acids and *A. oryzae* emulsin (Takadiastase) hydrolyze the dextrins completely to D-glucose.[125] Molecular weight determinations indicate that the β-dextrin is $(C_6H_{10}O_5)_6$ and the α-dextrin is $(C_6H_{10}O_5)_5$. Hydrolysis of the fully methylated dextrins yields 95% of 2,3,6-trimethylglucose and no traces of tetramethylglucose. The hydrolytic products contain no 2,3,4-trimethylglucose, as may be shown by tosylation of the glucosides formed from the hydrolysis products. Since none of the tosyl groups introduced are replaced by iodine on reaction with sodium iodide, there can be no free primary hydroxyls groups in the substances produced from the methylated dextrins[126] (see p 171). The small number of glucose units in the molecule is difficult to harmonize with an absence of end groups unless cyclic structures are present. Hence, it appears that these substances consist of cyclic rings of five glucose residues (α-dextrin) or six residues (β-dextrin) connected by maltose-type (1,1') linkages. Such structures agree with the evidence given, with the lack of reducing power and with X-ray diffraction studies. It should be noted, however, that according to French and Rundle these products contain six and seven glucose residues, respectively, rather than five and six. If so, they might be named as cyclohexaamylose and cycloheptaamylose.[127] However, such structures do not explain the large amount of iodine which is taken up in alkaline solution.

The cleavage of the rings by acids proceeds more rapidly than the subsequent hydrolysis of the linear chains. The linear chains formed in this manner are hydrolyzed by β-amylase.[128]

If the Schardinger dextrins exist preformed in the starch molecule, they

[123] F. Schardinger, *Zentr. Bakt. Parasitenk Infekt.*, [2] **22**, 98 (1908); **29**, 188 (1911)

[124] W. S McClenahan, E B. Tilden and C S. Hudson, *J. Am. Chem Soc*, **64**, 2139 (1942). For additional details of the preparation of the enzyme, see M Blinc, *Kolloid-Z.*, *101*, 126 (1942); *Chem Abst*, *38*, 4642 (1944)

[125] K Freudenberg and R Jacobi *Ann* *516*, 102 (1935)

[126] K Freudenberg and M. Meyer Delius, *Ber*, *71*, 1596 (1938)

[127] D. French and R. E. Rundle, *J Am Chem Soc*, **64**, 1651 (1942)

[128] G. T Cori, M A Swanson and C. F Cori, *Federation Proc*, 4, 234 (1945).

probably play an important part in the structure. As a result of this assumption it has been suggested that starch (amylopectin) may consist of five or six-membered closed rings with 24 to 30-membered side chains attached to each glucose residue of the rings.[129] However, the high yields of the dextrins makes it probable that they are products synthesized as a result of enzymic action and are not preformed in the starch molecules. Proof for this explanation is supplied by the formation of the dextrins in 70 percent yield from the straight-chain amyloses.[130]

"Synthetic" Starches and Phosphoamylases. As shown by Cori and Cori, animal tissues contain an enzyme which, through a mechanism involving phosphorylation, breaks down glycogen to glucose 1-phosphate (Cori ester)[131] The reaction is reversible, and the enzyme and similar enzymes from potatoes, peas and other sources act on the Cori ester to synthesize amylose- or glycogen-like polysaccharides.[132] One of these enzymes has been obtained in crystalline condition from rabbit skeletal muscle.[133] It crystallizes as a complex with adenylic acid. Although generally known as phosphorylases, a more descriptive name for the enzymes might be phosphoamylases.

The polysaccharide synthesized by Hanes using potato phosphorylase is remarkably similar to the amylose portion of the starch granule. The iodine color is more intense than that from ordinary starch, and solutions of the polysaccharide rapidly retrograde. The synthetic starch and amylose are reported to be quantitatively hydrolyzed by β-amylase to maltose. Similar X-ray diagrams are given by natural and synthetic starch.[134] The polysaccharide synthesized by muscle phosphorylase appears[135] to be closely related in structure and properties to that of Hanes. However, that obtained from glucose 1-phosphate by the action of liver, potato and yeast phosphorylases resembles glycogen or amylopectin in many of its properties.[136] According to Meyer and Bernfeld,[137] potato phosphorylase can hydrolyze only 1,4' linkages (maltose type), but yeast phosphorylase is able to cleave both 1,4' and 1,6' (isomaltose) bonds.

[129] K. Freudenberg, *Ann. Rev. Biochem.*, *8*, 81 (1939).

[130] R. W. Kerr, *J. Am. Chem. Soc.*, *64*, 3044 (1942); E. J. Wilson, Jr., T. J. Schoch and C. S. Hudson, *J. Am. Chem. Soc.*, *65*, 1380 (1943); M. Samec, *Ber.*, *75*, 1758 (1942)

[131] C. F. Cori, S. P. Colowick and G. T. Cori, *J. Biol. Chem*, *121*, 465 (1937).

[132] W. Kiessling, *Biochem. Z.*, *302*, 50 (1939); G. T. and C. F. Cori, *J. Biol. Chem.*, *135*, 733 (1940); C. S. Hanes, *Proc. Roy. Soc.* (*London*), *B128*, 421 (1940); *B129*, 174 (1940).

[133] G. T. and C. F. Cori, *J. Biol. Chem.*, *142*, 447 (1942).

[134] W. T. Astbury, F. O. Bell and C. S. Hanes, *Nature*, *146*, 558 (1940).

[135] W. Z. Hassid, G. T. Cori and R. M. McCready, *J. Biol. Chem.*, *148*, 89 (1943).

[136] R. S. Bear and C. F. Cori, *J. Biol. Chem.*, *140*, 111 (1941); W. N. Haworth, S. Peat and E. J. Bourne, *Nature*, *154*, 236 (1944).

[137] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, *25*, 399, 404 (1942).

An interesting catalytic effect of added starch substances has been observed for the reaction. When the enzyme and glucose 1-phosphate are mixed, a long induction period is observed unless small amounts of starch, glycogen or dextrin are added.[138] Adenylic acid is not required in the reaction.

A plausible explanation of the activating effect of polysaccharides on the enzymic synthesis of starch substances is that the activators serve as nucleii from which the starch chains are synthesized.[139] Crystalline muscle phosphorylase seems to act by the addition of glucose units (from glucose 1-phosphate) to the terminal groups present in the activators. This mechanism would explain the much greater activating effect of amylopectin and glycogen on the rate of synthesis as compared with amylose, for the former substances have a much greater number of terminal groups (per gram) than amylose. Although the original nucleus (activating substance) has a branched structure, the chains synthesized by the muscle phosphorylase probably are linear from the point of origin. It appears that the synthesis of linear chains does not continue indefinitely but stops when the chain reaches a length of about 200 glucose residues. Since amyloses already have a chain length of this degree of magnitude, they would be expected not to add many more glucose residues when subjected to the action of muscle phosphorylase in the presence of glucose 1-phosphate. Actually it was found that about 20 glucose units will add to an amylose chain originally 200 units in length.

The number of terminal groups in a unit weight of polysaccharide material increases as the material is hydrolyzed. Hence, it would be expected that the activation effect on the synthesis of starch substances by phosphorylases would increase with increasing degree of hydrolysis of the activator. This effect has been demonstrated for whole starch, amylose and amylopectin hydrolyzed by acids and by enzymes.[139, 140] The maximum activating effect is observed for materials having a chain length of about 5 glucose residues; shorter and longer chains are less effective.

Heart and liver tissue and potatoes contain a protein material, presumably an enzyme, which in conjunction with the crystalline muscle phosphorylase produces branched chains.[139, 141] This protein material apparently produces points of branching by the addition of glucose units to the 6-positions of existent straight chains. Thereafter, the muscle phosphorylases build up straight chains from the new branching points. This

[138] D. E. Green and P. K. Stumpf, *J. Biol. Chem.*, **142**, 355 (1942).

[139] G. T. Cori, M. A. Swanson and C. F. Cori, *Federation Proc.*, **4**, 234 (1945).

[140] P. H. Hidy and H. G. Day, *J. Biol. Chem.*, **152**, 477 (1944); **160**, 273 (1945); J. B. Sumner, G. F. Somers and E. Sisler, *ibid.*, **152**, 479 (1944); A. M. Kusin and V. I Ivanov, *Biokhimiya*, **10**, 37 (1945); *Chem. Abst.*, **39**, 3550 (1945).

[141] E. J. Bourne and S. Peat, *J. Chem. Soc.*, 877 (1945).

observation is of fundamental significance for it provides an explanation of the formation of the different types of polysaccharides (straight and branched chains) and also provides the opportunity of synthesizing materials of different degrees of branching.

No tetramethylglucose could be obtained from methylated synthetic amylose by Hassid and McCready, but Haworth, Heath and Peat have found an amount of tetramethylglucose (1.5%) corresponding to a chain length of 80 to 90 glucose members. As the viscosity of solutions of the methylated starch corresponds to the molecular weight of 80 to 160 glucose units, the synthetic starch seems to be a linear unbranched polymer of the amylose type.[142]

It is very striking that in living plants when starch is hydrolyzed it is sucrose, glucose and fructose which accumulate and maltose and dextrins are rarely found. Since it seems probable that the phosphorylases play the main role in this process, the relation of the phosphorylases to the well known amylases remains an important unsolved problem.

Certain writers have placed considerable stress on the presence of bound phosphorus in the starch components. It is of considerable interest, then, that Waldschmidt-Leitz and Mayer[143] find that a liquefying enzyme, free from α- and β-amylase and called amylophosphatase, may be obtained from barley by preferential adsorption on activated alumina and kaolin. The enzyme is said to liberate phosphate groups from corn starch at a rate proportional to the decrease of viscosity; however, there is very little maltose formation. The reducing power of the products of hydrolysis corresponds to a chain length of about 36 glucose units. This value is close to the average chain length of about 36 glucose units. It might seem from this evidence that the basic chains are connected by phosphoric ester linkages and that the amylopectins might be considered as consisting of basic straight chains of 24 to 30 glucose units connected by cross linkages formed
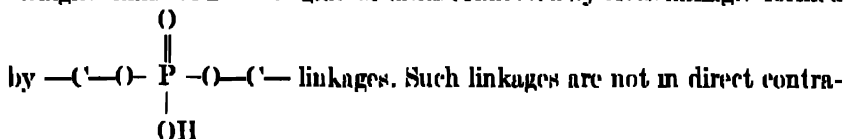
$$\text{by} \ —C—O— \overset{\displaystyle O}{\underset{\displaystyle OH}{\overset{\|}{\underset{|}{P}}}} —O—C— \ \text{linkages.}$$ Such linkages are not in direct contradiction to the glucosidic type of linkage usually postulated since the results of methylation studies might be similar for the two types of structures. The disaggregation caused by alkali might also be explained by such ester linkages, but the influence of oxygen on the process would not be clear. Also, the amount of phosphorus is too small to account for many such linkages.

[142] W. Z. Hassid and R. M McCready, *J. Am. Chem. Soc.*, **63**, 2171 (1941); W. N. Haworth, R L Heath and S Peat, *J. Chem. Soc.*, 55 (1942).

[143] E. Waldschmidt-Leitz and K Mayer, *Z. physiol. Chem*, **236**, 168 (1935).

It should be noted that Friedmann[144] was not able to find any phosphate liberated as the result of enzymic action. The existence of the amylophosphatase must be considered questionable until further evidence is obtained.

7. Industrial Preparation and Utilization of Starch and Starch Products.[145,1]

Although the physical properties of starches prepared from different sources vary considerably, for most industrial purposes it is possible to obtain suitable products from starch of any origin. An exception is the tapioca starch used for postage adhesives. In America most commercial starches are derived from corn, but in Europe the main source is potatoes. Raw starches are sold directly for household and industrial consumption or are converted to pastes, dextrins, sirups and dextrose. Ester and ether derivatives have not had much commercial application. The principal uses of starches and derived products are for textile and paper sizes, adhesives, food products, dusting powders, and the preparation of dextrose (D-glucose) and sirups.

Preparation of Potato Starch. Although in the early days of the industry a yield of 16 to 22% of starch was considered satisfactory, it has been possible by the development of special varieties of potatoes and of better growth conditions to obtain yields of 25 to 40%. After harvesting, the potatoes are washed and passed through a machine (consisting of a toothed cylinder revolving in a stationary cylinder) in order to rupture the cells containing the starch. To minimize enzymic action during the process, particularly the action of tyrosinase which forms dark bluish-black compounds, the pulped mass is treated with sulfur dioxide. For removing the starch, the pulp is passed over screens while water is sprinkled into the mass. The starch is washed through, and much of the fiber is retained on the screen. The starch suspension (containing starch, soluble matter, fiber and nitrogenous substances) is passed over inclined tables some 40 to 60 feet long, and the starch settles out during the transit of the suspension over the table. Purification is accomplished by stirring the deposited starch with water, filtering through silk cloth and repeating the tabling operation. This operation may be repeated several times or be replaced partially or entirely by centrifugation of the suspension. The final product on the refining tables is covered with a thin layer of colored impurities which is scraped off and

[144] S. Friedmann, *Enzymologia*, **6**, 307 (1939).

[145] J. A. Radley, "Starch and Its Derivatives;" Chapman & Hall, London (1943); W. B. Newkirk, *Ind. Eng. Chem.*, **31**, 153 (1939); J. P. Casey, *Tech. Assoc. Papers* (*TAPPI*), **25**, 139 (1942); R. W. Kerr, "Chemistry and Industry of Starch;" Academic Press, New York (1944).

returned to one of the earlier stages of the process. Since the product contains from 35 to 40% water, it is dried using temperatures from 30 to 45°C. before the final bolting and bagging.

Preparation of Corn Starch. Clean shelled corn is soaked in water containing SO_2 in large cylindrical tanks for 30 to 40 hours. The steeping not only washes the grains but softens the kernels so that they are more easily ruptured in the subsequent processes. In order to break up the kernels without injuring the germ, the corn is passed between two plates which have projecting teeth and which are rotating in opposite directions. The particles are washed through troughs with water; the lighter germ floats to the surface while the remainder of the grain settles out at the bottom. The germ is isolated and pressed free of oil, which after refining is sold as corn oil for cooking purposes. The heavier fraction is ground as finely as possible without rupturing the starch granules and is separated from the fibers by screening. The starch is separated and refined by tabling as described above for potato starch. In addition to the oil, a second important by-product is the gluten (protein) which is recovered from steep waters and from the effluent of the settling tables. The gluten may be sold as cattle feed or used in the preparation of plastics and other products. Commercial corn starch usually has a moisture content of 9 to 14%; in specially dried products, the amount of moisture may be as low as 5%.

Dextrins and British Gums. By enzyme, acid, heat and oxidation treatments, starches are partially hydrolyzed or oxidized to mixtures called dextrins, the composition of which depend on the extent of the degradation. Considerable skill is involved in the preparation of products having the desired properties. When dissolved in water, some yield thick pastes whereas others give thin, free-flowing liquids. The rate of drying of the pastes varies a great deal. Some solutions retain their viscosity and consistency over long periods of time while others retrograde rapidly. Hence, it is possible to prepare products of a wide range of properties, and for most purposes, special products are prepared.

Two general preparatory methods are often described as the wet and the dry processes. It is reported that the dry process was discovered as a result of a fire in a Dublin textile mill in which starch was stored. After the fire, it was found that the starch had been converted to a brown powder which dissolved in water to give a sticky solution. Products of this type, prepared by the high temperature treatment of starch with or without the addition of small quantities of acids or alkalies, are called "British gums" or "Torrefaction dextrins." To obtain practically colorless products (white dextrins and canary dextrins), the wet process is employed. The wet process consists of heating starch solutions with acids or with diastatic enzymes such as bacterial or malt extracts. These products as well as the oxidized starches

find widespread application as sizes for textiles and papers, as adhesives (pastes), as components of textile printing inks, etc.

The structure of dextrins prepared by the heat treatment of corn starch (British gums) has been investigated by Brimhall.[146] This light-brown material is water soluble and retains its granular structure. However, in contrast to the original starch, its X-ray pattern is of the amorphous type. Although corn starch contains 21% of amylose (determined by the iodine potentiometric method), the dextrins apparently contain none of the linear starch component. A possible explanation for the disappearance of the linear fraction is that the process of dextrinization involves the formation of cross links between starch chains. Such an explanation receives support from the increased resistance of the dextrins to hydrolysis by β-amylase. Thus, soybean β-amylase converts the dextrin to maltose in maximum yields of 22% although corn starch gives about a 55% yield of maltose. The end group content of the dextrins is considerably higher than that of corn starch.

8. Glycogen[147]

The important role played by glycogen as the reserve carbohydrate of animals makes it of great interest. It is found particularly in the muscle and liver cells of animals. It also occurs in insects and lower plants including fungi and yeasts. In contrast to starch, which occurs as discrete granules in plants, the glycogen is distributed throughout the protoplasm and is not morphologically differentiated. A portion of the glycogen contained in the cell is water soluble, but the remainder is said to be in combination with insoluble proteins. The separation from other cell constituents is facilitated by the stability of glycogen (in the absence of oxygen) to hot alkali which hydrolyzes most of the accompanying substances. Cold water dissolves the polysaccharide with the formation of opalescent solutions. With iodine, violet to brown colors are produced. The available evidence indicates that the term glycogen embraces a group of structurally related substances which are soluble in water and give a violet to brown color with iodine. As shown below, glycogen (or better glycogens) resembles amylopectin and, even more closely, the limit dextrin obtained from amylopectin by the action of β-amylase. Certain starches, e.g., sweet corn (*Zea mays*) and gluten rice (*Oryza glutinosa*) are known to contain glycogen-like fractions.[148]

Chemical Evidence for Structure. The evidence for the structure of

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[146] B. Brimhall, *Ind. Eng. Chem.*, *36*, 72 (1944).

[147] K. H. Meyer, *Advances in Enzymology*, *3*, 109 (1943).

[148] W. Z. Hassid and R. M. McCready, *J. Am. Chem. Soc.*, *63*, 1632 (1941); J. B. Sumner and G. F. Somers, *Arch. Biochem.*, *4*, 7 (1944); D. L. Morris and C. T. Morris, *J. Biol. Chem.*, *130*, 535 (1939).

glycogen is very similar to that for starch. Total hydrolysis with acids gives practically quantitative yields of D-glucose and proceeds through the intermediate formation of dextrins. Acetyl bromide produces hepta-acetylmaltosyl bromide in yields comparable to that obtained from maltose and starch. Thiethers and triesters are formed.

End group determinations reveal two types of glycogens; those with one terminal group for each 12 and those with one for each 18 glucose units.[149] These results indicate a more highly ramified structure for glycogen than for starch which has one terminal group for each 24 to 30 glucose residues. The yields of dimethylglucose, as would be expected, are equal to those of tetramethylglucose.
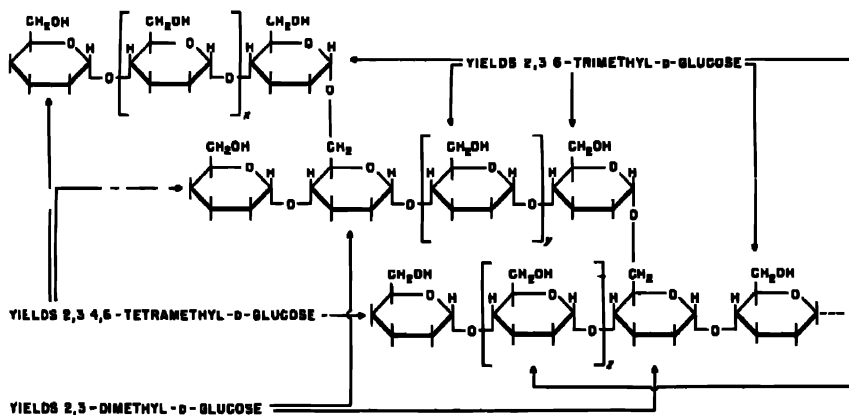
Fig. 4   Formula of glycogen (after Haworth).

Molecular Weight. Osmometric measurements on methylated and acetylated derivatives[150] disclose that glycogens prepared from a number of sources have molecular weights in the range 2.7×10^5 to 3.5×10^6. By repeated centrifugation of purified glycogen, a fraction is obtained with a molecular weight of 1,500,000 (osmometric method) and a degree of polymerization of 9,000. Photographs of this fraction made with the electron microscope show[151] that the particles have a diameter of 5 to 15 mμ. These values agree well with a particle size of about 10 mμ calculated from the observed molecular weight on the assumption of a cubic structure.

Bridgman[152] finds that glycogen preparations are very polydisperse and

[149] W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 577 (1937); W. N. Haworth, E. L. Hirst and F. Smith, *ibid.*, 1914 (1939); W. Z. Hassid and I. L. Chaikoff, *J. Biol. Chem.*, **123**, 755 (1938).

[150] S. R. Carter and B. R. Record, *J. Chem. Soc.*, 664 (1939).

[151] E. Husemann and H. Ruska, *J. prakt. Chem.*, [2] **156**, 1 (1940).

[152] W. B. Bridgman, *J. Am. Chem. Soc.*, **64**, 2349 (1942); K. H. Meyer and R. Jean-loz, *Helv. Chim. Acta.*, **26**, 1784 (1943).

that the molecular weights of different fractions vary from four to fourteen million. The molecules appear to be elongated and to have an axial ratio of at least 18 to 1.

Action of Enzymes. Malt β-amylase hydrolyzes glycogen much more slowly than soluble starch, and the hydrolysis stops after some 30% or the theoretical quantity of maltose has been formed.[163] Other investigators report that the hydrolysis is very incomplete but give different values for the resting stage. Glycogen from mussels, which has an end group ratio of 1 to 11, is reported to be hydrolyzed to the extent of almost 50% by purified β-amylase. Inasmuch as half of the total glucose residues are removed in the hydrolysis, the residual dextrin should have one end group for each 5.5 units. In confirmation of this deduction, the end group ratio is found to be 1:5.5 (18%).[164]

Structure. The evidence described above pictures the glycogen molecule as similar in its general outline to that for the amylopectin constituent of starches. From the end group determinations and the study of the β-amylase hydrolysis, it seems that glycogen is highly branched with the branches having five to seven glucose units each and with about three glucose units in the main chains between the branching points. Multiple branching is possible. The larger glycogen molecules may consist of many thousands of glucose units.

[163] G G Freeman and R H Hopkins, *Biochem. J.*, *30*, 446 (1936)
[164] K H Meyer and M Fuld, *Helv Chim Acta*, *24*, 375 (1941)

POLYURONIDES, HEMICELLULOSES, PLANT GUMS, MICROBIAL POLYSACCHARIDES AND RELATED SUBSTANCES[1]

In the present chapter, the polysaccharides other than cellulose, starch and glycogen are considered. The latter have been discussed in previous chapters because of their importance and because of the amount of material available. Those considered in the present chapter are not as well known, as well defined or as well studied as those in the previous chapters. Many of the materials discussed below have considerable present or potential importance. The immunological polysaccharides, in particular, are of great biological and medical interest. For the classification used, see Chapter XII.

1. Homopolysaccharides

A. Glucose Polymers. *Lichenin.* Lichenin occurs as an important cell-wall constituent of lichens, notably Iceland moss (*Cetraria islandica*) and apparently has a structure similar to that of cellulose. Its solubility in water is probably explained by its low molecular weight which is recorded as 10,000 to 37,000.[2] Acetolysis[3] leads to cellobiose octaacetate; complete hydrolysis produces glucose. In addition to trimethylglucose, about 0.9% of 2,3,4,6-tetramethylglucose is produced[4] by the acid hydrolysis of methylated lichenin; the yield of tetramethylglucose and the molecular weight determination agree with the assignment of a straight-chain structure to lichenin. As the X-ray diagram differs[5] from that of cellulose and as the derivatives are more levorotatory than the corresponding cellulose derivatives, it is possible that linkages other than the 1,4' type may be involved. Since a substance present in oats has the same action on the cupric chloride crystallization patterns as lichenin, the polysaccharide may be present in some higher plants.[6] Some evidence exists for ascribing the enzymic hydrolysis of lichenin to lichenases, different from cellulases (see under Cellulases)

Bacterial Cellulose. When grown on sugar solutions, *Acetobacter xylinum* produces membranes of cellulose. Fructose, glycerol and mannitol provide

[1] General references: K. H. Meyer, "High Polymers," Vol 4, 347; Interscience Publishers, New York (1942) A. G. Norman, "The Biochemistry of Cellulose, the Polyuronides, Lignin, etc."; Oxford (1937); *Ann. Rev. Biochem.*, *10*, 65 (1941). E Anderson and L. Sands, *Advances in Carbohydrate Chem.*, *1*, 329 (1945).

[2] S. R Carter and B. R. Record, *J Chem. Soc.*, 664 (1939)

[3] P. Karrer and B. Joos, *Biochem. Z.*, *136*, 537 (1923).

[4] K. Hess and L. W Lauridsen, *Ber.*, *73*, 115 (1940).

[5] R. O. Herzog, *Z. physiol. Chem.*, *152*, 119 (1926).

[6] D. L. Morris, *J. Biol. Chem.*, *142*, 881 (1942).

excellent substrates; many other carbohydrates, except pentoses, also may be employed. The viscosities of solutions of the bacterial cellulose, as well as the X-ray patterns and the properties of the acetates and methylated derivatives, indicate that the substance is closely similar to cotton cellulose.[7]

Examination[8] of the cellulose membranes by use of the electron microscope shows that they are composed of discrete, intermeshed fibers about 200 $\mathring{A}$ in width and 100 $\mathring{A}$ in thickness.

Yeast Dextran.[9] A yeast polysaccharide, unaffected by the action of hot dilute acids and alkalies and originally called "yeast cellulose" by Salkowski, appears to have an unusual 1,3' polymeric linkage. The methylated dextran yields 2,4,6-trimethylglucose and enough 2,3,4,6-tetramethylglucose to account for one end group for each 28 glucose residues. Inasmuch as the products of hydrolysis exhibit an upward mutarotation, it is believed that the glucosidic linkages have the beta configuration. Oligosaccharides obtained by partial acid hydrolysis are hydrolyzed by almond emulsin. The viscosity of solutions of the methylated polysaccharide indicates a molecular weight of about 6500. Although the structure appears closely similar to that of cellulose except for the position of the glucosidic linkages, the properties of the two substances are significantly different. Yeast dextran does not dissolve in ammoniacal copper oxide solutions and does not produce cellobiose octaacetate by acetolysis.

Glycogen and yeast mannan, discussed elsewhere, accompany the dextran as constituents of yeast but are more soluble and may be preferentially extracted.

Bacterial Dextrans. Dextrans are synthesized from sucrose, but not from glucose, by certain bacteria (genus, *Leuconostoc;* family, *Coccaceae*). Three species of *Leuconostoc* are distinguished according to their ability to ferment xylose and L-arabinose as well as sucrose (*L. mesenteroides*), or sucrose but not the pentoses (*L. dextranicus*), or neither pentoses nor sucrose (*L. citrovorus*). The dextrans are obtained by growing the bacteria on solutions containing sucrose and nutrients.[10] Afterwards, the culture medium is evaporated, and the dextran is precipitated by the addition of alcohol. The synthesis of the polysaccharide from sucrose through the agency of enzymes present in bacteria-free filtrates of *Leuconostoc* cultures has been reported.[11]

[7] J. Barsha and H. Hibbert, *Can. J. Research, 10,* 170 (1934).

[8] E. Franz and E. Schiebold, *J. macromol. Chem., 1,* 4 (1943).

[9] W. Z. Hassid, M. A. Joslyn and R. M. McCready, *J. Am. Chem. Soc., 63,* 295 (1941); L. Zechmeister and G. Tóth, *Biochem. Z., 84,* 133 (1936); V. C. Barry and T. Dillon, *Proc. Roy. Irish Acad., 49B,* 177 (1943).

[10] H. L. A. Tarr and H. Hibbert, *Can. J. Research, 5,* 414 (1931); W. Z. Hassid and H. A. Barker, *J. Biol. Chem., 134,* 163 (1940); M. Stacey, *Nature, 149,* 639 (1942); W. L. Owen, Jr., and W. L. Owen, U. S. Patent 2,392,258, Jan. 1, 1946.

[11] E. J. Hehre and J. Y. Sugg, *J. Exptl. Med., 75,* 339 (1942); M. Stacey, *loc. cit.*

Three dextrans, obtained in this manner, are distinguished by Hibbert and associates by the suffixes I, II and III. Two (I, II) are produced by strains of *L. mesenteroides* and one (III) by *L. dextranicus*.

Methylated dextran-I is hydrolyzed to tetramethylglucose, 2,3,4-trimethylglucose and 2,3-dimethylglucose in the relative proportions of 1:3:1. The experimental details and probable structure are discussed in an earlier section (p. 519).

Hydrolysis of methylated dextran-III gives 90% yields of 2,3,4-trimethylglucose and 10% of dimethylglucose. Although Fairhead, Hunter and Hibbert could find no tetramethylglucose, a small amount (0.23%) later was obtained.[12] The basic structure of the dextran-III molecule is a chain of 1,6' linked α-glucopyranose residues. The formation of dimethylglucoses indicates branching to be present providing the methylation is complete, but since the position of the methyl groups has not been determined, the location of the branches is not known. Osmotic pressure measurements show that the molecule must contain at least 200 glucose units.

A dextran synthesized by a strain of *L. mesenteroides* has been studied by Hassid and Barker. It seems to be similar to the dextran-II of Hibbert and associates and to be composed of α-glucopyranose residues with 1,6' polymeric linkages. Viscosity measurements indicate a molecular weight of 11,700 while the ultracentrifuge (sedimentation equilibrium method) gives a value of 2600 ± 50.

The dextrans form precipitates not only with *L.* antisera but also with types 2, 12 and 20 pneumococcal antisera.[11]

A very similar dextran is synthesized by *Betabacterium vermiformé* from sucrose, but it has a much smaller repeating unit since 1 to 5% of tetramethylglucose is produced along with 90% of 2,3,4-trimethylglucose.[13] As the degree of polymerization is about 500 (osmotic pressure method), the molecule must contain 20 repeating units each of which consists of 25 α-glucopyranose residues

Photographs of one of the *L. mesenteroides* dextrans by use of the electron microscope show a branched, thread-like structure.[14] The chains have a thickness of about 50 Å. Since the length of a glucose chain is about 5 Å, the threads could be composed of central linear chains with side chains of about 5 glucose residues.

B. Fructose Polymers.[15] Fructosans or levans are found widely dis-

[12] E. C. Fairhead, M. J. Hunter and H. Hibbert, *Can J Research, B16,* 151 (1938); S. Peat, E. Schluchterer and M. Stacey, *J. Chem. Soc.,* 581 (1939).

[13] W. D Daker and M. Stacey, *J Chem. Soc.,* 585 (1939).

[14] B. Ingelman and K. Siegbahn, *Nature, 154,* 237 (1944).

[15] H. K. Archbold, *New Phytologist, 39,* 185 (1940); E J McDonald, *Advances in Carbohydrate Chem., 2,* 253 (1946)

tributed throughout the plant kingdom, particularly in the *Compositae* and related families, and generally serve as reserve polysaccharides in place of, or in addition to, starch. Their distribution in the monocotyledons has been investigated.[15] Grasses contain considerable quantities at the time of heading, but thereafter the fructosan content decreases during maturation. Smaller quantities are found in cereals. The most important and most investigated of the fructosans is inulin discovered in 1804 by Rose. This polysaccharide occurs in Jerusalem artichokes, chicory, burdock, golden-rod, dandelion, and dahlia plants. It is best prepared from dahlia tubers or chickory roots.[17] The heated and neutralized juices are filtered and allowed to crystallize in the cold. Although inulin may be prepared from the other plants listed, the yields are low because of the presence of many similarly constituted polysaccharides which are more soluble and probably of smaller particle size. The high fructosan content of many easily grown but otherwise practically useless plants has stimulated much work on the preparation of crystalline levulose or levulose sirups from these sources (see D-Fructose).

Hydrolysis of inulin by enzymes leads to a practically quantitative yield of levulose (D-fructose) although a small quantity of glucose also may be produced.[18a,b] The inulases (which may be identical with invertases) are found in fungal enzyme preparations and particularly in *Aspergillus niger* emulsins.[18a, 19] Yeast invertase preparations also hydrolyze inulin.[18b, 20] Acid hydrolysis takes place with about the same ease as that of sucrose but with the production of about 92% fructose, 3% glucose and 5% of a group of three nonreducing difructose anhydrides. The latter substances, as shown by the careful investigations of Jackson, McDonald and Goergen, are apparently reversion products, the structures of which have been previously considered (see p. 216).

Methylated inulin gives on hydrolysis principally 3,4,6-trimethylfructose. With the assumption of furanose rings, which assumption seems correct because of the ease of hydrolysis, the polymeric linkages must exist between carbons 1 and 2' of adjacent residues. Accompanying the tri-

[15] A. G. Norman, *J. Am. Soc. Agron.*, *31*, 751 (1939), A. S. Morosov, *Compt. rend. acad. sci. U.S.S.R.*, *24*, 407 (1939).

[17] See. F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars"; Circular 440 of the National Bureau of Standards; p 398 (1942); J J Willaman, *J. Biol. Chem.*, *51*, 275 (1922). The latter reference gives much historical material concerning the polysaccharide.

[18a] H. Pringsheim and P. Ohlmeyer, *Ber.*, *65*, 1242 (1932).

[18b] M. Adams, N. K. Richtmyer and C S. Hudson, *J. Am. Chem Soc.*, *65*, 1369 (1943).

[19] W. W. Pigman, *J. Research Natl. Bur Standards*, *30*, 150 (1943).

[20] R. Weidenhagen, *Z. Ver. deut Zucker Ind.*, *82*, 316, 912 (1932).

methylfructose is about 3.7% of 1,3,4,6-tetramethylfructose. This quantity corresponds to one end group for 27 to 30 fructofuranose residues.[21]

Inulin
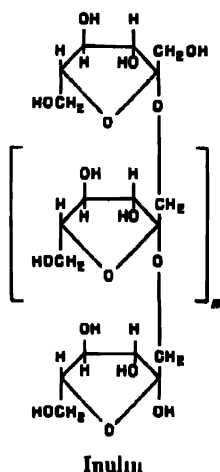
Smaller yields of the tetramethylfructose are reported by Irvine and Montgomery whereas Schlubach and Sinh[22] were unable to find any trace of this product. The absence of any tetramethylfructose in products of hydrolysis of methylated inulin was taken as evidence for a closed chain structure.

Other fructosans of plant and bacterial origin have been investigated. Schlubach[24] and associates, who have made special contributions to the chemistry of these polysaccharides, classify them into two general types: (1) those with 1,2' linkages (inulin group) and (2) those with 2,6' linkages (phlein group). Phlein is the fructosan obtained from the tubers of the grass *Phleum pratense*. Asparagosin, sinistrin and graminin yield, after methylation and hydrolysis, 3,4,6-trimethylfructose and belong to the inulin group. They give greater amounts of tetramethylfructose than inulin and also considerable quantities of dimethylfructose. Hence, they are considerably more branched than inulin. The 1,3,4-isomer is the only trimethylfructose obtained from secalin, poäin, and phlein (from plants) and from bacterial levan. The latter is produced from sucrose and raffinose by *B. subtilis*, *B. mesentericus* and other bacteria, as well as by the cell-free enzyme preparations.[23][24] The principal polymeric linkage of this group

[21] W. N. Haworth, E. L. Hirst and E. G. V. Percival, *J. Chem. Soc.*, 2384 (1932); J C. Irvine and T. N. Montgomery, *J. Am. Chem. Soc.*, 55, 1988 (1933).

[22] H. H. Schlubach and O. K. Sinh, *Ann.*, 544, 105 (1940).

[24] H. H. Schlubach and O. K. Sinh, *Ann.*, 544, 101, 111 (1940).

[23] H. Hibbert, R. S. Tipson and F. E. Brauns, *Can. J. Research*, 4, 221 (1931); R. R. Lyne, S. Peat and M. Stacey, *J Chem. Soc.*, 237 (1940); S. Hestrin, S. Avineri-Shapiro and M. Aschner, *Biochem. J.*, 37, 450 (1943); S. Hestrin and S. Avineri-Shapiro, *ibid.*, 38, 2 (1944).

must be of the 2,6' type, if the probable assumption is again made that the rings are of the furanose type. Phlein probably consists of a closed loop of fructofuranose residues since the sole product of methylation and hydrolysis is the 1,3,4-trimethylfructose

The position of irisin (obtained from the rhizomes of *Iris pseudoacorus*) in the above classification is questionable since the hydrolysis products of the methylated irisin consist of equimolecular quantities of 1,3,4,6-tetramethylfructose and 3,6(?)-dimethylfructose.[25] If the identification of the dimethylfructose is correct, a new type of connection (2,4') between residues is present. The absence of any trimethylfructose and the equality in the amounts of the tetramethyl and dimethylfructoses can mean only that the irisin is completely branched since every fructose unit is either an end group or attached to an end group. A possible structure is illustrated.
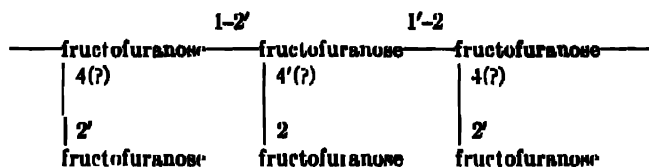
(Possible structure of irisin)

C. Galacturonic Acid Polymers (Pectins).[26] Fruits, berries and tubers contain a group of polysaccharides called the pectic substances. Although initially present in the plant in an insoluble state probably in combination with unknown constituents of the cell wall, they are solubilized by treatment with dilute acids (or enzymes), and pectins are obtained from the solution by precipitation with alcohol or 50% acetone. Such a treatment is likely to yield products contaminated with other plant constituents. This contamination probably provides the explanation for the reports of early workers in the field that the pectins yield not only D-galacturonic acid on hydrolysis but also D-galactose and L-arabinose. In addition to these hydrolysis products, Ehrlich also reports the presence of acetic acid and methyl alcohol.[27]

Later work has demonstrated that the pectins are composed of D-galacturonic acid or D-galacturonic acid methyl ester residues. Schneider and Bock[28] found that associated galactose- and arabinose-containing materials could be separated from the pectins by careful fractional precipitation with alcohol. Also, no evidence could be found for the presence of acetyl groups.

[25] H. H. Schlubach, H. Knoop and M. Liu, *Ann.*, *504*, 30 (1933).

[26] C. L. Hinton, "Fruit Pectins," Chemical Publishing Co., New York (1940); E. L. Hirst and J. K. N. Jones, *Advances in Carbohydrate Chem.*, *2*, 235 (1946).

[27] F. Ehrlich and F. Schubert, *Ber.*, *62*, 1974 (1929).

[28] G. G. Schneider and H. Bock, *Ber.*, *70*, 1617 (1937).

Speiser, Eddy and Hills [29] have studied the rate of removal by acids of nonuronide material associated with pectins. The activation energy for the process (18,500 cal.) is that to be expected for the rupture of primary covalent bonds. It is possible, then, that the natural pectic substances may have polygalacturonide chains esterified with arabans or galactans but that the latter can be removed to a considerable degree and leave intact chains of galacturonic acid residues. Similar types of linkages have been suggested for the cell-wall components of woody tissue (see under Hemicelluloses).

The nomenclature of the pectic substances and the related enzymes used in the present discussion follows that suggested by the Committee on Nomenclature of Pectin of the Division of Agricultural and Food Chemistry of the American Chemical Society.[30] The definitions for pectic substances are quoted below.

"Pectic Substances. 'Pectic substances' is a group designation for those complex colloidal carbohydrate derivatives which occur in or are prepared from plants and contain a large proportion of anhydrogalacturonic acid units which are thought to exist in a chainlike combination. The carboxyl groups of polygalacturonic acids may be partly esterified by methyl groups and partly or completely neutralized by one or more bases.

"Protopectin. The term 'protopectin' is applied to the water-insoluble parent pectic substance which occurs in plants and which upon restricted hydrolysis yields pectin or pectinic acids.

"Pectinic Acids. The term 'pectinic acids' is used for colloidal polygalacturonic acids containing more than a negligible proportion of methyl ester groups. Pectinic acids, under suitable conditions, are capable of forming gels with sugar and acid or, if suitably low in methoxyl content, with certain metallic ions. The salts of pectinic acids are either normal or acid pectinates.

"Pectin. The general term 'pectin' (or pectins) designates those water-soluble pectinic acids of varying methyl ester content and degree of neutralization which are capable of forming gels with sugar and acid under suitable conditions.

"Pectic Acids. The term 'pectic acids' is applied to pectic substances mostly composed of colloidal polygalacturonic acids and essentially free from methyl ester groups. The salts of pectic acids are either normal or acid pectates."

Pectins from different sources exhibit marked differences in jellying power, ash, methoxyl content and other properties, as shown in Table 1, and hence probably represent a group of substances rather than a single

[29] R. Speiser, C. R. Eddy and C. H. Hills, *J. Phys. Chem.*, *49*, 563 (1945).

[30] *J. Am. Chem. Soc.*, *49*, No. 5, 37 (Proceedings) (1927), *Chem. Eng. News*, *22*, 105 (1944).

substance. Schneider and Bock[28] ascribe these variations in the properties
of pectins from various sources to differences in molecular size, in the degree
of esterification of the carboxyl groups and in the amounts and types of
accompanying polysaccharide impurities.

Hinton[29] has studied the properties of a number of fruit pectins, and the
accompanying table gives the maximum variations noted.

Because of the presence of non-galacturonide materials in many pectin
preparations, the percentage of polygalacturonide material and the degree
of esterification (as per cent) are more significant properties than percentage
of methoxyl groups.[31]

The enzymic hydrolysis[32] of pectins gives crystalline D-galacturonic acid
in yields of as much as 85% although acid hydrolysis gives lower yields

TABLE I

Properties of Fruit Pectins

(after Hinton)

Property	Range
Jellying power	0–29 5
Free acidity	1.3 10.3 g NaOH/100 g pectin
pH of 1% solutions	2 6–4.4
Methoxyl content	9–12% CH_3OH
Saponification value	7.6–15 1 g. NaOH/100 g. pectin
Total acidic groups	16 7 to 19 6 g. NaOH/100 g. pectin
Iodine reduction power	2.7–23.3% reducing sugar (as glucose)
Ash	0 59–8.90%

presumably as a result of decarboxylation of the uronic acid (p. 308). The
production of such large quantities of galacturonic acid, in conjunction
with the analyses given above, is evidence that the pectins are polygalac-
turonic acids. The linkages do not involve the carboxyl groups as the total
amount found for such groups (after removal of metallic ions and methyl
groups) is in agreement with that calculated on the assumption of a free
carboxyl for each uronic acid residue. The ease of removal of the methyl
groups by dilute alkalies shows that they are esterified with the carboxyl
groups in the original pectins. As shown by the above analyses, however,
the number of esterified carboxyl groups is extremely variable.

[31] C. H Hills and R. Speiser, *Science*, *103*, 166 (1946).

[32] F. Ehrlich, "Abderhalden's Handbuch der biologischen Arbeitsmethoden,"
Abt. 1 , Teil 11, 1617 (1936); H. H. Mottern and H. L. Cole, *J. Am. Chem. Soc.*,
61, 2701 (1939); W. W. Pigman, *J. Research Natl. Bur. Standards*, *25*, 301 (1940);
H. S. Isbell and H. L. Frush, *ibid.*, *32*, 77 (1941); *33*, 389 (1944); C. S. Hollander, U. S.
Patent 2,370,901, March 6, 1945.

The demethylated pectins are known as pectic acids and their salts as the pectates. Some of the pectates, e.g., the calcium pectates, are so insoluble that they are used for the quantitative estimation of pectins. Pectinic acid has a single buffer range. It does not behave as a monobasic acid, for at a given pH, the degree of dissociation increases with increasing concentration and with increasing degree of esterification by methyl groups.[31]

Pectins are extremely difficult to methylate but by repeated treatments using first the thallium hydroxide-methyl iodide and then the silver oxide-methyl iodide method, fully methylated methyl esters of strawberry and citrus pectins have been obtained.[34] Methanolysis at elevated temperature and pressure yields as the main product methyl 2,3-dimethylgalactofururonide.

Mild acylation of commercial pectin with pyridine and acid anhydrides gives disubstituted esters. Pectin dibutyrate and dipropionate films are of moderate strength and flexibility whereas that from the diacetate is very weak.[35]

The presence of the furan ring in the product of methanolysis cannot be taken as evidence for such a ring in the original pectin as the conditions of methanolysis are such that ring shifts can take place. Since 2,3-dimethylgalacturonic acid forms furanosides under the conditions used in the methanolysis of the methylated pectin, either type of ring may have been present in the original material. However, the high dextrorotations of pectins and their resistance to acid hydrolysis is such that pyranoside rings probably are present.[31] According to this evidence, the pectins are composed of chains of galacturonic acids connected through 1,4' linkages probably having the alpha configuration.
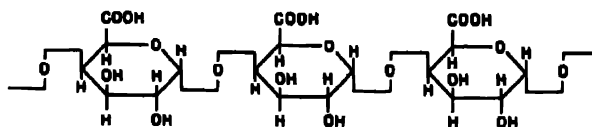
Pectic acid

Morell, Baur and Link[36] treated citrus polygalacturonide with methyl alcoholic hydrogen chloride (glycoside-forming conditions) and obtained a series of degraded polygalacturonic acid methyl esters. In this process, free reducing groups of the polygalacturonides are converted to methyl glycoside groupings and carboxyl groups are esterified. The esterified methyl

[31] R. Speiser, C. H. Hills and C. R. Eddy, *J. Phys. Chem.*, **49**, 328 (1945).

[34] G. H. Beaven and J. K. N. Jones, *Chemistry & Industry*, **58**, 363 (1939); S. Luckett and F. Smith, *J. Chem. Soc.*, 1106, 1114, 1506 (1940).

[35] J. F. Carson, Jr., and W. D. Maclay, *J. Am. Chem. Soc.*, **67**, 787 (1945)

[36] S. Morell, L. Baur and K. P. Link, *J. Biol. Chem.*, **106**, 1 (1934).

groups were removed by treatment with alkali, which treatment leaves the glycosidic methyl groups intact. A methoxyl analysis gave a value of 2.24%, which indicates the presence of one glycosidic methyl group for each 8 to 10 galacturonic acid residues. This product is undoubtedly degraded as is the methylated pectin of Luckett and Smith[34] which according to osmotic pressure measurements contains 13 galacturonic acid residues.

Citric pectic acid or citrus polygalacturonide is a commercial product made by the acid extraction of citrus pulp.[37] The acid extract is treated with calcium and sodium hydroxides which precipitate the calcium pectate. Treatment of the latter product with acids gives the citrus pectic acid. The latter contains 95 to 99% of galacturonic acid and is ash-free. It is reported that different batches of this material vary considerably. The industrial applications and preparations of pectic substances have been reviewed by Baier and Wilson.[38]

The molecular weights of the polysaccharides of fruit juices (mainly pectins) have been determined from ultracentrifugal studies on the juice and on the alcohol-precipitable material.[39] Although not monodisperse, the products vary less in molecular weight than cellulose and starches. Apple, pear and plum pectins exhibit molecular weights falling in the interval 25,000 to 35,000, but orange pectins give values in the range 40,000 to 50,000. Osmometric and viscometric determinations[28,10] on carefully nitrated pectins, however, give molecular weights falling in the interval 100,000 to 200,000 for fruit pectins, about 20,000 to 25,000 for beet pectins, and 3,000 to 30,000 for flax pectins. When the pectin-containing plant material is nitrated directly, the isolated pectin has a higher molecular weight than the product obtained by nitrating the isolated pectin. Hence, it would appear that the intact protopectins are of higher molecular weight than those isolated by the usual procedures. Since fruit pectins usually occur free in the juices and require but little chemical treatment to accomplish their isolation, they would be expected to be of higher molecular weight than root and leaf pectins which require more drastic isolation conditions.

Henglein[41] has pointed out that pectins found free in solution (fruit and berry juices) have a greater proportion of ester methoxyl groups than those in the cell walls of plants. He suggests that the free carboxyl groups act as bridges between pectin chains and possibly carboxyl groups of other cell

[37] K. P. Link and R. Neddon, *J. Biol. Chem.*, *94*, 307 (1931–32).
[38] W. E. Baier and C. W. Wilson, *Ind. Eng. Chem.*, *33*, 287 (1941)
[39] The Svedberg and N. Gralén, *Nature*, *142*, 261 (1938).
[40] H. Bock and R. Einsele, *J. prakt. Chem.*, [2] *155*, 225 (1940); see, however· H. S Owens, H. Lotzkar, T. H. Schultz and W. D. Maclay, *J. Am. Chem. Soc.*, *68*, 1628 (1946).
[41] F. A. Henglein, *J. makromol. Chem.*, *1*, 121 (1943).

wall constituents such as cellulose, arabans, galactans, etc. Divalent ions such as Ca^{++}, Mg^{++}, etc., may hold two carboxyl groups together by ionic linkages in a manner similar to their behavior in the complex silicates. Since the number of binding groups would increase as the number of unesterified carboxyl groups increase, difficultly soluble pectins would be expected to have only a small methoxyl content as appears to be the case.

The number of free carboxyl groups greatly affects the strength of gels formed in the presence of sucrose and calcium salts, for the main types of bonding in the gel is ionic ($-CO-O$ $Ca-OCO$). On the other hand, sucrose-pectin gels depend for their structure on hydrogen bonding between pectin molecules and the sugar, and the strengths are most dependent upon the molecular weight of the pectin material.[42]

Although pectins do not give good X-ray patterns, those produced from sodium pectate are satisfactory.[43] For oriented fibers of sodium pectate, the fiber axis identity period is 13.1 Å, somewhat smaller than the corresponding value for cellulose (15.2 Å). The chains have the configuration of a threefold screw axis, and pseudohexagonal symmetry is exhibited.

Pectic Enzymes.[44] Enzymes exist in many natural products which act on pectins. Those present in fungi, bacteria and malt have received the most study. There is considerable interest in these enzymes as agents for the clarification of fruit juices and for the preparation of D-galacturonic acid. Large quantities of an *Aspergillus* enzyme preparation (emulsin) are made particularly for the former purpose.[45]

According to the Committee on Nomenclature of Pectin,[10] the pectic enzymes are defined as:

Protopectinase. "The term applied to the enzyme which hydrolyzes or dissolves protopectin with the resultant separation of the plant cells from each other, usually spoken of as maceration."

Pectase. "The term applied to the enzyme which converts pectin into pectic acid, the latter becoming a gel, especially in the presence of calcium (or barium or strontium) salts."

Pectinase. "The term applied to the enzyme which hydrolyzes pectin and pectic acid into their simplest soluble cleavage products, ..."

The enzyme dissolving the middle lamella is called protopectinase. The presence of this type of enzyme in fungi, bacteria and plants has been noted. Davison and Willaman,[44] in a survey of a number of materials, re-

[42] R. Speiser and C. R. Eddy, *J. Am. Chem. Soc.*, *68*, 202 (1946).

[43] K. J. Palmer and M B Hartzog, *J. Am. Chem. Soc*, *67*, 2122 (1945); K. J Palmer, *J. Applied Phys.*, *17*, 405 (1946)

[44] F. R. Davison and J. J. Willaman, *Botan. Gaz.*, *83*, 329 (1927); Z. I. Kertesz, *Ergeb. Enzymforsch.*, *5*, 233 (1936), F Ehrlich, *Enzymologia*, *3*, 185 (1937).

[45] Rohm and Haas "Pectinol." (U. S. Patent 1,932,833, Feb. 27, 1931.) A German preparation for the same purpose is called "Filtragol."

ported the presence of the enzyme in the extracts of a number of fungi. Almond emulsin and "Takadiastase" also were found to exhibit this type of activity.

Pectinases are also widely distributed in fungal and plant products.[44] The first enzyme of this type, present in barley malt, was reported by Bourquelot and Hérissey.[46] *Asperyillus oryzae* emulsin ("Takadiastase") contains a pectinase but *Rhizopus tritici*, *Sclerotinia cincrea* and *Bacillus carotovorus* emulsins provide better sources. The pH optimum of the enzymes lies near 3.0 and inactivation is rapid at 60°C. Certain species of *Penicillium* are also excellent sources. Commercial "Pectinol," however, is reported to be an *Aspergillus* emulsin. It is of interest that certain of the *Penicillia* have little or no amylase action although, as mentioned, they are good sources of pectic enzymes. Pectinase (also called by Ehrlich pectolase) is probably the most important of the pectic enzymes, since it is responsible for the hydrolysis of the glycosidic linkages between the galacturonic acid residues. As mentioned previously, yields as high as 85 per cent of crystalline D-galacturonic acid are obtained from pectins by the action of this enzyme.

Pectinases may be separated from pectases (see below) by adsorption on cation-exchange resins.[47]

Pectase (called pectin-methoxylase by Kertesz) hydrolyzes the ester linkages of pectins with the liberation of methyl alcohol and pectic acid. It appears to be very widely distributed in nature and is frequently found in the roots, leaves and berries of higher plants. It is often found free of pectinase. Although quite active for the hydrolysis of the ester groups of pectins and degraded polygalacturonides, these enzymes have very little hydrolytic action on the galacturonic acid methyl ester. The enzyme appears to have no optimal pH, but the activity increases continuously as the pH increases until the enzymic hydrolysis is replaced by alkaline saponification. The activity is determined by the measurement of the amount of free carboxyl groups formed after 30 minutes under standard conditions.[48]

In the initial phases of the action of enzymes on pectins, a marked decrease in viscosity takes place accompanied by only a small increase in reducing power. This effect does not seem to be related to the removal of methyl groups since a similar decrease in viscosity with no increase in the reducing power takes place when pectins are heated at 100°C. at pH 3.2 Under the latter conditions no cleavage of methyl ester groups takes place. These results are interpreted as meaning that the pectin molecule consists of an aggregate of polygalacturonic acid chains of the form $[(G)_m]_n$ in

[44] E. Bourquelot and H. Hérissey, *J. pharm. chim.*, [6] 8, 145 (1898).

[47] R. J. McColloch and Z. I. Kertesz, *J. Biol. Chem.*, 100, 149 (1945)

[48] Z. I. Kertesz, *J. Biol. Chem.*, 121, 589 (1937)

which the polygalacturonic acid chains are represented by $(G)_n$. The preliminary action of the enzymes is to break down the aggregates into short chains.[49]

It is not necessary to assume that the initial rapid reduction in viscosity produced as a result of enzymic or acid hydrolysis is due to the existence of weak bonds in the pectin chains. A sharp drop in the weight-average molecular weight is to be expected in the early stages of hydrolysis.[50] Also the activation energy in the initial stages is that required for the cleavage of ordinary glycosidic linkages (ca. 28,000 cal. per mole.)[51]

D. Polymers of Other Hexoses, Pentoses and Uronic Acids.[52] *Mannans.* Two types of mannans have been distinguished: those with 1,4' and those with 1,6' polymeric linkages.[53] Members of the first group appear to be structurally analagous to cellulose and, on the basis of comparisons of optical rotations, to have β-glycosidic linkages To this group belong mannans A and B of the ivory nut (*Phytelepas macrocarpa*) as well as the salep mannan found in the *Orchidaceae* and probably pine mannan. Yeast mannan (yeast gum), which also contains 1,2' connections and mannocarolose, which is produced by the fungus *Penicillium charlesii* from glucose solutions, belong to the group with 1,6' linkages. A gluco-mannan, Konjak mannan, obtained from the corms of *Conophallus konjak*, has similar 1,6' connections.

Ivory Nut Mannans. The high mannose content of the endosperm of the ivory nut makes this material valuable for the preparation of mannose (see under Mannose). The extraction of ivory nut shavings with 5% sodium hydroxide solution dissolves mannan-A and with 10% alkali, the mannan-B.[54] The methylated mannans-A and B, on acid hydrolysis, yield 2,3,6-trimethylmannose and small amounts of 2,3,4,6-tetramethylmannose (identified as the anilides). The proportions of end-groups found agree with a chain length of 70–80 for the more soluble mannan-A. The average molecular weight is about 13,000.[55]

Salep Mannan. Orchid tubers (*orchis* sp.) yield an alcohol-precipitable mannan when extracted with cold water. As methylation and hydrolysis produce the same products as the ivory-nut mannans, the polymeric linkage is mainly of the 1,4' type. The end-group content agrees with a chain length of 70 to 80 units.[55] Osmometric and viscometric measurements of salep mannan fractions prove that the product is inhomogeneous and that

[49] Z. I. Kertesz, *J. Am. Chem. Soc.*, *61*, 2544 (1939).
[50] R. Speiser and C. R. Eddy, *J. Am. Chem. Soc.*, *68*, 287 (1946).
[51] R. C. Merrill and M. Weeks, *J. Am. Chem. Soc.*, *67*, 2244 (1945).
[52] E. Husemann, *J. prakt. Chem.*, [2] *155*, 13 (1940).
[53] F. Klages and R. Maurenbrecher, *Ann.*, *535*, 175 (1938).
[54] M. Lüdtke, *Ann.*, *456*, 201 (1927).
[55] F. Klages and R. Niemann, *Ann*, *523*, 224 (1936)

the degree of polymerization is as much as 20 times greater than the size of the repeating unit as determined from the end group determination. This difference may be due to degradation during the methylation reaction or to a comparison of different fractions of the mannan. Branching of the molecule seems less probable since the viscosities of salep mannan solutions are about the same as those of degraded cellulose solutions of the same degree of polymerization.[52] This evidence indicates that the mannan molecules are linear polymers.

Pine Mannans. In contrast to the negligible mannan content of the Angiosperms, the Gymnosperms yield mannans in quantities as high as 10%. In order to obtain the mannans, alkaline extraction, following a pretreatment with chlorine dioxide, is employed. Methylation studies have not been carried out on these mannans, but as their properties are very similar to those of ivory-nut mannan-A, the linkages possibly are also of the 1,4' type. Since osmometric and viscometric studies give results similar to those for salep mannan, pine mannans appear to be linear polymers closely related to cellulose.[54] The purity of these products is doubtful.

Yeast Mannan By the extraction of yeast with hot water, Schutzenberger obtained a polysaccharide which he precipitated from solution by the addition of alcohol. Higher yields result from the use of strong acids or alkalies as the extracting agents. Complete hydrolysis of methylated mannan leads to 2,3,4,6-tetramethylmannose (2 moles), 3,4,6-trimethylmannose (1 mole), 2,4,6-trimethylmannose (1 mole), 3,4-dimethylmannose (2 moles) and small quantities (<3%) of 2,3,4-trimethylmannose. The nature of the products proves the existence of three types of linkages: 1,6', 1,2' and 1,3', in the relative proportions 2:3:1. Various combinations of these polymeric linkages are possible, but the basic repeating unit consists of six mannopyranose units with two of the six in terminal positions.[55] A possible structure is illustrated:

```
   1'-2          1-3'         1'-2          1-2'
--- mannose  — mannose    mannose--- mannose—
          |                       |
          6                       6
          |                       |
          1'                      1'
      mannose                 mannose
```
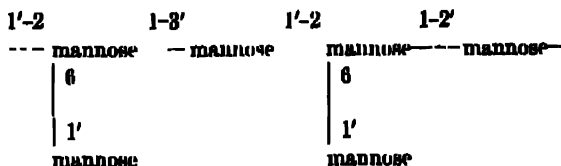
(A possible repeating unit of yeast mannan)

Mannocarolose. A polysaccharide mixture is produced when *Penicillium charlesii* grows on glucose solutions. After fractional precipitation with alcohol, two polysaccharides are obtained one of which, called mannocarolose, yields mannose and the other of which gives galactose after

[54] W. N. Haworth, R. L. Heath and S. Peat, *J. Chem. Soc.*, 833 (1941).

acid hydrolysis. The polymeric connection of the mannan is of the 1,6′ type since 2,3,4-trimethylmannose results from methylation and subsequent hydrolysis. Enough 2,3,4,6-tetramethylmannose (13.4%) is also produced to account for a repeating unit or molecule of 9 mannose units.[57] The capsular substance of *Bacillus krzemieniewski* has also been identified as a mannan.[58]

Konjak Mannan. Superheated water (110–125°C.) will extract a gluco-mannan from the corms of *Conophallus konjak*. The same product is produced as the result of the action of pancreas preparations on Konjak meal. Total hydrolysis leads to glucose and mannose in the relative proportions of 1:2. The methylated polysaccharide yields on hydrolysis 2,3,4-trimethylmannose, 2,3,6-trimethylmannose and 2,3,4-trimethylglucose. Hence, the polymeric linkages appear to be of the 1,6′ and 1,1′ type.[59] By acetolysis of Konjak mannan, a trisaccharide consisting of two mannose and one glucose unit is obtained.

Konjak flour is used in Japan as a foodstuff.

Mannuronic Acid Polymers.[60] Dilute alkalies extract from brown sea weeds (*Phaeophyceae*) (see Fig. 3, p. 632) a polysaccharide called algin or alginic acid which has some industrial interest because of its property of forming viscous mucilaginous solutions and because of its possible use as a textile fiber. Solutions of alginic acid are acidic and decompose carbonates. The neutralization equivalent falls in the range 176 to 184. Acid hydrolysis to the constituent uronic acids is difficult to achieve because of simultaneous decarboxylation, but by the action of 80% sulfuric acid for 5 days at room temperature hydrolysis to the extent of about 80% is effected. From the products of hydrolysis, the cinchonine salt of D-mannuronic acid is obtained.[61]

Methylation and hydrolysis of a degraded alginic acid (obtained by boiling sodium alginate with 10% hydrogen chloride in methanol) yields the methyl ester of methyl 2,3-dimethylmannuronide. Hence, the polymeric linkages probably are of the 1,4′ type as is evidenced by the stability of the polysaccharide to acid hydrolysis; the β-configuration for the linkages seems probable because of the large levorotation of the polysaccharide. Since periodic oxidation of 2,3-dimethyl*manno*saccharic acid produces glyoxylic acid and in addition dimethyl-L-erythruronic acid, the glycosidic unions

[57] W. N. Haworth, R. Raistrick and M. Stacey, *Biochem. J.*, **29**, 612 (1935).

[58] A. Kleczkowski and P. Wierzchowski, *Soil Science*, **49**, 193 (1940).

[59] K. Nishida and H. Hashima, *Bull. Agr. Chem. Soc. Japan*, **8**, 54 (1932); T. Otuki, *J. Chem. Soc. Japan*, **60**, 1181 (1939), **61**, 531 (1940).

[60] A. G. Norman, "The Biochemistry of Cellulose, Etc.," *loc. cit.*,[1] p. 142.

[61] W. L. Nelson and L. H. Cretcher, *J. Am. Chem. Soc.*, **54**, 3409 (1932); S. Miyake and K. Hayashi, *J. Soc. Trop. Agr. Taihoku Imp. Univ.*, **11**, 95, 204 (1939); G. M. Bird and P. Haas, *Biochem. J.*, **25**, 403 (1931).

must involve carbons 4 or 5.[62] The direct oxidation of alginic acid by periodic acid would be expected to produce a 2,3 dialdehyde from each mannuronic acid residue, and this dialdehyde should be oxidized to a tricarboxylic acid. On acid hydrolysis, the dialdehyde should yield D-erythruronic acid and glyoxal, and the bromine oxidation product should give *meso*-tartaric acid and glyoxylic acid. Experimentally it is found that 42% of the theoretical quantity of glyoxal and 25% of *meso*-tartaric acid are obtained from the corresponding products of periodic acid oxidized alginic acid. These results provide supplementary evidence that the linkages cannot involve the hydroxyls of carbons 2 and 3.[63]

Alginic acid has become of considerable interest as a possible textile fiber. It dissolves in alkaline solutions and may be spun into filaments which are said to have a greater stability to acids than cellulose filaments. Such filaments are not suitable for practical purposes since they dissolve in alkaline solutions. The aluminum, calcium, barium, chromium and particularly the beryllium salts of alginic acid are insoluble in water and may be of value for making commercial fibers. The high content of carboxyl groups should be advantageous in delustering processes since large quantities of metallic ions can be taken up, and these frequently promote delusterification. Such products also are extremely noninflammable.

Galactans. In addition to agar and the water-soluble arabo-galactan of larch wood which are discussed later, the galactan occurring in the albumin glands of the vineyard snail (*Helix pomatia*) has received considerable study.[64] This galactan is accompanied by glycogen and is known as snail galactogen. Methylation and acid hydrolysis of snail galactogen produce approximately equal amounts of 2,3,4,6-tetramethylgalactose and 2,4-dimethylgalactose but no trimethylgalactose. These results are only interpretable on the basis of a highly branched structure in which every galactose unit either is an end group or is attached to an end group.

```
        1-6'              1'-6            1-6'
 — — galactose— —galactose- ---galactose-    galactose-- —
     3                                          3'


    .1'            .1              .1'         | 1
galactose      galactose       galactose      galactose
```

(Possible repeating unit of snail galactogen)
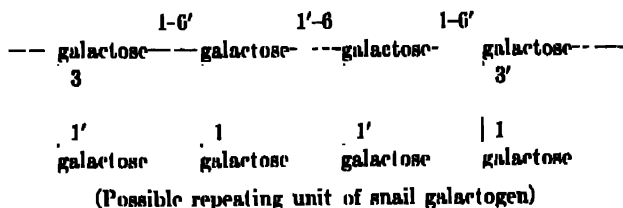
Although such a high degree of branching is unusual, a similar compound composed of fructofuranose units (irisin) is known. Since some D,L-galac-

[62] E. L. Hirst, J K N. Jones and W. O. Jones, *J Chem Soc* , 1880 (1939)

[63] H. J Lucas and W. T. Stewart, *J. Am. Chem. Soc.*, 63, 1070, 1792 (1940).

[64] H. H. Schlubach and W. Loop, *Ann* , 532, 228 (1937); E. Baldwin and D. J. Bell, *J. Chem. Soc.*, 1461 (1938).

tose occurs in the products of hydrolysis, at least one of the units of the above structure is an L-galactose residue.[65]

Pentosans. Peanuts (*Arachis hypogaea*) yield as a result of mild alkaline hydrolysis an araban-pectic acid complex from which an araban may be extracted by 70% ethyl alcohol. Approximately equal proportions of 2,3,5-trimethyl-L-arabinofuranose, 2,3-dimethyl- and 3-methyl-L-arabinose are produced by acid hydrolysis of the methylated araban.[66] The trimethylarabinose must have a furanose structure. In analogy, as well as from the ease of hydrolysis of the araban, it is assumed that all of the arabinose residues in the polysaccharide have furanose structures. The trimethylarabinose fraction must represent terminal groups, the monomethyl-L-arabinose points of branching, and the dimethyl-L-arabinose unbranched positions in the chains. Several possible repeating units of the structure are illustrated. Arabans from citrus and apple pectins seem to be similarly constituted.

```
5               1-5'          1'        2                1'
-arabinofuranose----arabinofuranose----, or   - arabinofuranose ----
              | 2'                              | 5
              | 1                               | 1'
              arabinofuranose                   arabinofuranose
                                                | 5'
                                                | 1
                                                arabinofuranose
```
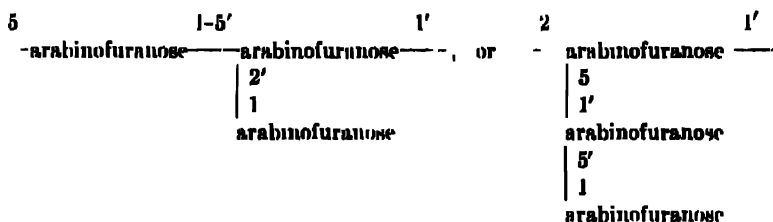
(Possible repeating units of peanut araban)

A xylan occurring in esparto grass contains small quantities of L-arabinofuranose residues. Since the latter are easily removed by the action of 0.2% oxalic acid at 100°C. they must be located at the ends of the chains. The xylan is represented as continuous chains of about 19 xylose units each, terminated by arabinofuranose residues and cross-linked through the reducing group of one chain and a hydroxyl (carbon 3?) of another.[67]

The oxidation of wood and straw xylan by periodic acid has been studied. The process may have some value for the production of 2-carbon and 3-carbon hydroxy acids and polyglycols.[68]

Wood xylans have not been isolated in a high degree of purity. One of the purest of such materials is a fraction obtained from the holocellulose of aspen (*Populus*) wood and contains 85% of xylose residues.[69]

[65] D. J. Bell and E. Baldwin, *J. Chem. Soc.*, 125 (1941).
[66] E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 496 (1938); G. H. Beaven, E. L. Hirst and J. K. N. Jones, *ibid.*, 1865 (1939).
[67] R. A. S. Bywater, W. N. Haworth, E. L. Hirst and S. Peat, *J. Chem. Soc.*, 1983 (1937).
[68] G. Jayme and M. Sätre, *Ber.*, 75, 1840 (1942); 77, 242, 248 (1944).
[69] B. B. Thomas, *Paper Ind. and Paper World*, 27, 374 (1945)

E. Polymers of Glucosamine (Chitin). A unique nitrogen-containing polysaccharide (chitin) composes the organic skeletal substance of insects, crustacea and fungi, but it probably does not appear in the higher plants. Although extremely resistant to hydrolysis, chitin is hydrolyzed by concentrated acids to equimolecular quantities of glucosamine and acetic acid and by enzymes to N-acetylglucosamine.[70] From the products of partial hydrolysis of chitin, a disaccharide (chitobiose) has been isolated as the acetate.[71] It is probably 1-glucosamine D-glucosaminide.

Although conclusive evidence for the structure of chitin is lacking, it is probable that the polysaccharide consists of continuous chains of N-acetyl-glucosamine residues connected through 1,4′ β-glucosidic linkages. Such a structure is strictly analogous to that of cellulose and differs principally in the substitution of the NH—CO—CH₃ group for the hydroxyl group at carbon 2 of each glucose unit in the cellulose chain.
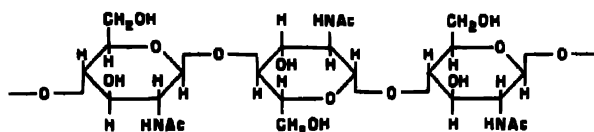
Chitin chain

The assignment of a structure for chitin analogous to that for cellulose receives support from X-ray diffraction studies.[72] The unit cell contains eight N-acetylglucosamine residues, is orthorhombic and has the following dimensions:

$$a = 9.40\ \text{Å}; \qquad b = 10.46\ \text{Å}; \qquad c = 19.25\ \text{Å}.$$

All the evidence available indicates that chitin from animal sources is identical with that from fungi.[73]

The insolubility of the polysaccharides makes the molecular weight determination difficult. The determination has been accomplished by dissolving native chitin in 50% nitric acid, measuring the viscosity at various times thereafter, and then extrapolating to zero time. Chitin from several sources is found in this manner to have about the same degree of polymerization as wood cellulose under the same conditions.[74]

[70] G. Ledderhose, *Ber.*, **9**, 1200 (1876); H. Brach, *Biochem. Z.*, **38**, 468 (1911); P. Karrer and G. v. Francois, *Helv. Chim. Acta*, **14**, 986 (1920).

[71] M. Bergmann, L. Zervas and E. Silberkweit, *Ber.*, **64**, 2436 (1931).

[72] K. H. Meyer and G. W. Pankow, *Helv. Chim. Acta*, **18**, 589 (1935); G. L. Clark and A. F. Smith *J. Phys. Chem.*, **40**, 863 (1936).

[73] J. M. Diehl and G. van Iterson, Jr., *Kolloid-Z.*, **73**, 142 (1935).

[74] K. H. Meyer and H. Wehrli, *Helv. Chim. Acta*, **20**, 353 (1937).

2. Heteropolysaccharides Derived from Several Sugar Types[7a—z]

Polysaccharides which are hydrolyzed to several monosaccharide types frequently are encountered. They are of considerable interest to the biochemist. Although their industrial importance is small at present, they should receive additional investigation from economic considerations alone since some are very plentiful and are by-products of commercial processes, e.g., of wood cellulose purification. Although the investigation of the structures of these substances has hardly been more than commenced, it is possible to write incomplete structures for some of the compounds. In most instances, however, the complexity of the structures is such that unequivocal formulas cannot be proposed. The problems involved in structural studies are difficult, and even the basis for the purification of the naturally occurring mixtures or complexes remains to be established. The present method often involves the use of hydrolytic agents to bring the substances into solution and the use of selective precipitants for purification. Such a procedure leaves much to be desired and makes it practically impossible to know much about the combinations present in the original untreated material.

A. Hemicellulose and Cell-wall Polysaccharides. Woody tissues contain carbohydrates, lignin, organic extractives (tannins, terpenes, alkaloids, fats, sterols, etc.) and inorganic salts. The carbohydrates are the principal constituent, with the lignin next in quantity. The organic extractives are of considerable importance; their nature is one of the characterizing properties of various woods. The extractives usually are removed by treatment of woody tissue with hot alcohol and ether.

An organic material called lignin which is rich in aromatic rings is present in considerable amounts. The lignin may be combined chemically with the carbohydrates of the cells. Both its chemical composition and genesis are unsettled.[7b] The material is of considerable potential importance because large quantities are available as a by-product of the preparation of paper pulp and of other processes. Tentative structures for lignin have been proposed by Freudenberg and by Hibbert. The material may be a polymer in which the basic unit is a phenylpropane radical:

[7a] A. G. Norman, "The Biochemistry of Cellulose, the Polyuronides, Lignin, etc.", Oxford (1937), *Ann. Rev. Biochem.*, *10*, 65 (1941).

[7b] K. H. Meyer, "High Polymers," Vol. *4*, p. 347; Interscience Publishers, New York (1942).

[7c] E. Hägglund, 'Holzchemie," Akademische Verlagsgesellschaft, Leipzig (1939).

[7d] L. E. Wise (Editor), "Wood Chemistry," Reinhold, New York (1944); A.C.S. Monograph 97.

[7e] E. L. Hirst, *J. Chem. Soc.*, 70 (1942).

[7f] For discussions of the subject see: H. Hibbert, *Ann. Rev. Biochem.*, *11*, 185 (1942); M. Phillips, *Chem. Revs.*, *14*, 103 (1934); E. G. V. Percival, *Ann. Repts. Prog. Chem.*, *39*, 142 (1942).
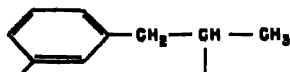
Numerous procedures have been suggested for the removal of lignin from the carbohydrates of the cell wall. That for Cross and Bevan "cellulose" represents one of the earliest and best known methods.[77] This method involves the treatment of moist, finely divided, extractive-free wood alternatively with chlorine and with sulfurous acid and sodium bisulfite until the lignin is removed, and a white residue (Cross and Bevan cellulose) is obtained. This type of procedure seems to have been described first by Fremy and Terreil.[78] Although this treatment removes the lignin, a considerable quantity of carbohydrate material is lost in the process.

Chlorine dioxide (chlorous acid) removes lignin and has considerably less degradative action on carbohydrates than chlorine. One of the first products made by the use of this reagent was called *Skelettsubstanz* (skeletal substance) by E. Schmidt. Alternate treatment of woody tissue with chlorine and with an alcoholic solution of pyridine[79] or ethanolamine[80] yields *holocellulose*, a similar product. In the ethanolamine process some of the amine reacts with hemicellulose groups and is retained in the holocellulose fraction. For this reason, the method has been modified[81] to a treatment of wood meal with aqueous sodium chlorite and acetic acid at 70 to 80° C. The holocellulose obtained in this way is white and retains the original woody structure. It contains small amounts of lignin (2.8 to 3.5%) which can be removed only at the expense of a loss in the more soluble carbohydrate (hemicellulose) fraction. The method has particular value for the preparation of large amounts of material for structural studies.

According to the Cross and Bevan definition, the α-cellulose content of Cross and Bevan cellulose obtained from woody tissues is the material insoluble in cold 17.5% sodium hydroxide. The β-cellulose consists of the material precipitated from the alkaline extracts by the addition of acids, and the γ-cellulose comprises the portion remaining in the acidified extracts. These definitions are extremely arbitrary, and under other conditions the amounts of the three fractions are different. The α-cellulose fraction agrees closely with the cellulose of Chapter XIII. The alkali-soluble material consists of polysaccharides and some lignin, the latter with some

[77] C. F. Cross and E. J. Bevan, *J. Chem. Soc.*, **38**, 666A (1880); "Cellulose," p. 95, Longmans, Green & Co., London (1910).

[78] E. Fremy and Terreil, *Bull. soc. chim.*, [2] **9**, 430 (1868).

[79] E. Schmidt and associates, *Cellulosechem.*, **12**, 201 (1931); **13**, 129 (1932). See also summary by A. G. Norman, *loc. cit.*[76a]

[80] W. G. Van Beckum and G. J. Ritter, *Paper Trade J.*, **104**, No. 19, 49 (1937).

[81] L. E. Wise, M. Murphy and A. A. D'Addieco, *Paper Trade J.*, **122**, No. 2, 35 (1946); G. Jayme, *Cellulosechem.*, **20**, 43 (1942).

of the polysaccharide material being precipitated by acids. The polysaccharides which dissolve in the alkaline solution are termed hemicelluloses. The hemicelluloses are defined by Norman as "those cell-wall polysaccharides which may be extracted from plant tissues by treatment with dilute alkalies, either hot or cold, but not with water and which may be hydrolyzed to constituent sugar and sugar-acid units by boiling with hot, dilute mineral acids." Of the hemicelluloses, the components closely associated with structural cellulose are designated *cellulosans*. An additional separation of hemicelluloses into an A-fraction, precipitated by acidification of the alkaline extracts, and a B-fraction, precipitated from the alkaline extracts only by the addition of acid and alcohol, is made by some investigators. The A- and B-fractions subsequently mentioned refer to this procedure unless noted otherwise.

Wise, Murphy and D'Addieco[51] obtained hemicellulose-A and -B by extraction with 4 to 5% and 24% potassium hydroxide, respectively. The carbohydrate material in the extracts was precipitated by means of acetic acid and ethanol. The procedure is particularly applicable for analytical determinations as all of the fractions are obtained in a solid condition.

The term hemicellulose was originated by E. Schultze (1892) who considered that this material might be intermediate in the formation of cellulose. Since the alkali-soluble fraction is hydrolyzed by acids to D-xylose, D-galactose, L-arabinose and uronic acids in addition to D-glucose, the term appears inapt but likely to be continued. The relationship of these various substances is illustrated in Fig. 1.

Wood pulp as prepared by the usual commercial practices contains considerable quantities of hemicelluloses. As shown by Jayme,[52] the presence of hemicelluloses in wood pulp used for paper is generally beneficial, and pulps high in α-cellulose produce papers with poorer mechanical properties than those with appreciable amounts of hemicelluloses. Apparently the latter materials act as bonding agents for the paper fibers.

Since the removal of lignin, hemicelluloses and cellulosans from the cell walls of several types of wood has no marked influence on the X-ray pattern,[53] it is probable that the lignin lies between and not within the micelles.

The hemicelluloses from the English oak have been extensively investigated by O'Dwyer. A schematic diagram of the separations is given in Fig. 2. Fractions precipitated from the alkaline extracts by the addition of acids (hemicellulose-A) were prepared from sap-wood and heart-wood. The main sugar obtained on acid hydrolysis of both fractions is D-xylose. In the

[52] G. Jayme, *Papier-Fabr. Wochbl. Papierfabr.*, **223**, 295 (1944); *Chem. Abst.*, *40*, 3895, 3897 (1946).
[53] R. D. Preston and A. Allsopp, *Biodynamica*, *3*, No. 53 (1939)

A-fractions, the xylose is combined with uronic and methoxyaldobiuronic acids. A difference between sap- and heart-wood is shown by the formation of about 10% glucose from the sap-wood fraction by the action of *Aspergillus oryzae* emulsin (Takadiastase) while the other fraction gives no
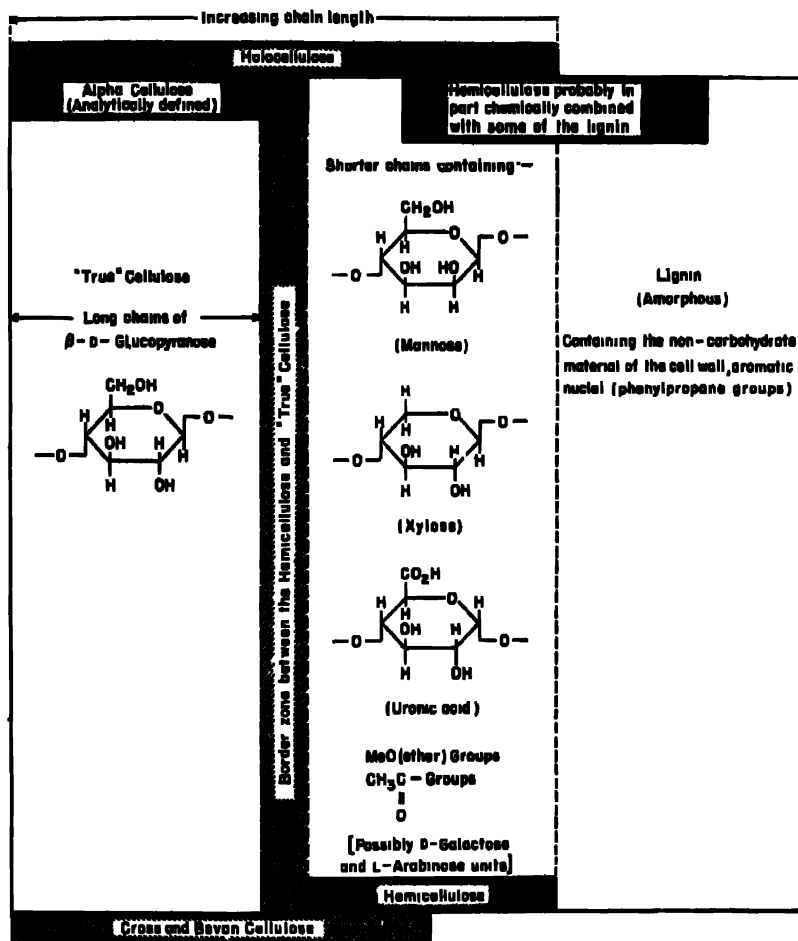
Fig. 1 Components of the extractive-free plant cell wall. (After L. E. Wise)

glucose. The enzyme-resistant polysaccharides from the sap- and heart-wood fractions are apparently identical; and prolonged additional treatment with the *A. oryzae* emulsin leads to the formation of a soluble polysaccharide and three moles of xylose. This soluble polysaccharide consists of six moles of xylose and one mole of a monomethylhexuronic acid.

The alcohol-precipitable material (hemicellulose-B) from the acidified solutions also seems to contain a structural unit consisting of six xylose

Extracted Oakwood

Treat with 4% aqueous NaOH

Filter

Solution of Hemicellulose   Wood residue

Treat with acetic acid

Solution of Hemicellulose

Filter, treat with ethanol

Precipitate of Hemicellulose A, which contains

82% anhydroxylose, 10% methyluronic acid; 8% anhydroglucose
Gives blue color with iodine

Precipitate of Hemicellulose B, which contains 60% anhydroxylose 17% methyluronic acid and 23% anhydroglucose (dextro-rotatory)

Treat with Takadiastase until coloration with iodine ceases

Hemicellulose Residue
$\{[\alpha]_D$ (in 2% NaOH) $-97.5°\}$

D Glucose in solution

Long continued treatment with Takadiastase

93–94% enters solution

3 Parts Xylose   2 Parts of a water soluble polysaccharide

$[\alpha]_D$ $-51°$

Heated 3 hours with 1% H_2SO_4

5 moles Xylose   1 mole "Aldobiuronic Acid"

Fig. 2. O'Dwyer's separation and stepwise hydrolysis of hemicelluloses from oak-sapwood.

residues and one methylhexuronic acid residue. The same difference in the glucose content exists for the sap-wood and heart-wood preparations as for the A-fractions, and it appears that the main change of composition which takes place in the transformation of sap-wood to heart-wood consists in the removal or transformation of glucose residues. The greater solubility of the B-fractions is probably to be ascribed to the greater amounts of uronic acids present.

The composition of the hemicellulose fractions obtained from hardwoods of different types also have been investigated by Anderson and associates,[53a] who report that they usually consist of a series of xylose units associated with a monomethylhexuronic acid, presumably methylglucuronic acid, which has an ether rather than an ester-linked methoxyl group. Glucose is often found in the hydrolytic products with the xylose and may be a component of the hemicellulose molecules or may be derived from associated molecules such as starch or cellulose dextrins. Hemicellulose from the compression wood of the white pine contains some mannose as well as xylose units, and that from the normal white pine wood contains glucose as well as mannose and xylose residues. In general, it appears that in the various hardwoods, approximately 7 to 19 xylose units are associated with one molecule of the uronic acid, which is combined with the neighboring xylose unit through a glycosidic linkage involving the reducing group of the uronic acid. Since the hemicelluloses originally are obtained by alkaline extraction but not by extraction with water and thereafter are water soluble, it seems probable that originally they are connected to some cell constituent in the intact wood through an ester linkage involving the carboxyl group of the uronic acid. Because the lignin is also solubilized by the treatment, it may be one of the constituents to which hemicellulose is attached.

The holocellulose of maple wood has been separated into four fractions by extraction with increasingly stronger solvents in the order: boiling water, 2% cold sodium carbonate solution, 4% cold sodium hydroxide solution and boiling 10% sodium hydroxide solution.[54] In Table II, the composition of the various fractions is given. The molecular weights were determined by the iodine titration method of Bergmann and Machemer, which, as previously explained, may not give reliable results. The molecular weights of the fractions increase with the insolubility of the fractions. The main difference in the fractions seems to be that the proportion of xylose increases with apparent molecular weight and at the expense of the hexose fraction.

Other lignified tissues have a similar composition. Wheat straw hemicellulose is principally of the B-type, and hexuronic acid, L-arabinose and

[53a] See summary: E. Anderson, R. B. Kaster and M. G. Seeley, *J. Biol. Chem.*, *144*, 767 (1942).

[54] R. L. Mitchell and G. J. Ritter, *J. Am. Chem. Soc.*, *62*, 1958 (1940).

xylose in the relative proportions 1:0.9:23 are found in the hydrolysate. The alkali-soluble fraction of alfalfa hay contains 12.1% uronic acid anhydride (probably the monomethylhexuronic acid) and 77.3% pentosan, which is mainly composed of xylose units although some L-arabinose residues are also present.[55] Cotton seed hull hemicellulose[56] consists of D-glucuronic acid and D-xylose in the approximate ratio of 1 to 10-16.

The hemicellulose of New Zealand flax (*Phormium tenax*) has been partially investigated by methylation methods.[57] The methylated hemicellulose upon methanolysis gave methyl 2,3,4-trimethylxyloside (about 11%), methyl 2,3-dimethylxyloside and the methyl ester of a methylated polyuronide containing xylose and glucuronic acid residues. The 11% of trimethylxylose represents end groups; approximately one of each ten residues is a terminal xylose group, and the structure is highly branched.

Fraction	Min Mol. Weight	Approx. Moles in One Mole of Hemicellulose					
		Uronic Acid	Xylose	Hexose	Methoxyl	Acetyl	Total
IA	1,070	1	4	2	1	2	10
IB	2,250	2	8	3	2	5	20
II	3,830	6	16	3	3		28
III	10,500	7	63	4	7		81

Osmotic pressure studies indicate that components of the xylan fractions obtained from wheat straw and beech wood and the mannan from spruce have degrees of polymerization falling in the range 120-200 units as compared with a minimum value of 1500 for beech-wood cellulose. The viscosities of solutions of the xylans and mannans are of a magnitude similar to those of cellulose preparations of similar degrees of polymerization. This correspondence provides evidence that all of these substances are linear polymers.[52]

Several monosaccharides and uronic acids may be present in the hydrolyzates from hemicelluloses, but commonly glucose, xylose and glucuronic acid or galactose, L-arabinose and galacturonic acid are found in the same hydrolyzates. This type of occurrence might be expected since the individual members of each of these two groups are closely related from the structural standpoint. By the oxidation of the primary hydroxyl groups of a member of the cellulose homologous series, a polyglucuronic acid would be

[55] H. D. Weihe and M. Phillips, *J. Agr. Research*, *60*, 781 (1940); M Phillips and B L. Davis, *ibid.*, *60*, 775 (1940).

[56] E. Anderson J. Hechtman and M. Seeley, *J. Biol. Chem*, *130*, 175 (1938).

[57] R. J. McIlroy, C. S. Holmes and R. P. Mauger, *J Chem. Soc.*, 796 (1945).

produced which in turn by decarboxylation would pass to a xylan. A similar oxidation of a galactan would yield a polygalacturonic acid, and decarboxylation would give an L-araban. Although this origin of pentosans is appealing and may apply to hemicelluloses, it does not explain the presence of arabinofuranose units in peanut araban or the association of arabinofuranose and xylopyranose residues in esparto xylan. Also, a galactan, isolated from the pectin constituent of seeds of *Lupinus albus*, consists of a chain of β-D-galactopyranose units, whereas an accompanying araban seems to be composed of L-arabinofuranose residues.[7b] Certain bacteria are reported to have the power to decarboxylate glucuronic acid to xylose, but the establishment of the presence of the corresponding enzymes in plants requires further investigation. Peculiarly enough, although mannose is

TABLE III

Composition of Wood Hydrolyzates

Sugar	(Deciduous) Birch	(Coniferous) Jack Pine
	%	%
Total reducing sugar	70.8	68.7
D-Glucose	47.9	46.4
D-Galactose	0.0	4.3
D-Mannose	1.3	9.7
D-Xylose	21.3	6.1
L-Arabinose	0.3	2.2

found as a hydrolysis product of certain hemicellulose fractions, the presence of the corresponding uronic acid and pentose (mannuronic acid and lyxose) has not been demonstrated.

Saccharification of Wood.[58] Treatment of wood with strong acids at elevated temperatures saccharifies the constituent polysaccharides. The sugars found in the hydrolyzate are dependent upon the type of wood. Considerable quantities of D-xylose are produced along with D-glucose from deciduous woods. Smaller quantities of pentose result from the hydrolysis of coniferous woods, but considerable amounts of D-mannose and often D-galactose are formed. The composition of hydrolyzates of jack pine and birch, determined at The Institute of Paper Chemistry after hydrolysis by 72 % sulfuric acid followed by dilution and boiling, is reported in Table III. Such hydrolyzates have been used for the production of ethyl alcohol by fermentation processes. In order to utilize the pentoses, special organisms such as *Torula datilla* and *Fusarium* species, have been used. Sometimes the production of alcohol is secondary to the production of protein material

[58] E. C. Jahn, "Wood Chemistry," Editor: L. E. Wise, p. 774; Reinhold, New York (1944); E. Hägglund, "Holzchemie," p. 255; Akademische Verlagsgesellschaft, Leipzig (1939).

for fodder purposes. For the latter purpose, conditions are chosen favorable for the growth of the organism rather than for its maximum conversion of fermentable carbohydrates to alcohol. Waste liquors from the sulfite process for paper making have similar compositions and also may be used.

In both World War I and II, wood hydrolyzates were used as a source of fermentable sugar.[89] The two most widely known processes are the so-called Scholler and Bergius processes. The Scholler process has been favored in the United States, and several plants using it were built in Germany, Italy, Manchuria and Korea in the decade preceding World War II. The process as carried out in Germany, involves hydrolysis of wood chips with 0.5% sulfuric acid under a pressure of 165-180 pounds per square inch. At the end of about forty-five minutes the dilute sugar solution is cooled, neutralized, and otherwise prepared for fermentation to alcohol. This process is the outgrowth of a method patented by Simonsen in 1894.[90] Several other processes using dilute acid, including one using dilute sulfurous acid, have been used at times.[91]

The Bergius process uses 40 to 42% hydrochloric acid. The action of the strong acid on the glucose which is formed, its corrosive effect on the equipment and the difficulties encountered in its recovery contribute to the difficulties involved in the use of this process. No successful development of this process in the United States has been reported.[92]

Arabo-galactan. From the wood of the western larch (*Larix occidentalis*), as well as that of other larches, an arabo-galactan may be obtained by extraction with water.[93] According to the definition previously cited for the hemicelluloses, this product is not to be included with the hemicelluloses since it is originally water-soluble and does not require a preliminary treatment with alkali. However, because of the structural similarities, it seems desirable to consider it at this point. Although the properties of fractions obtained by fractional precipitation of solutions of the arabo-galactan and of derivatives exhibit appreciable differences, and indicate that the polysaccharide is a mixture, it seems probable that the various fractions are structurally related.

As a result of studies of the products of methylation and partial hydrolysis of arabo-galactan fractions, White has provided information sufficient for the establishment of a tentative structure. Total acid hydrolysis gives D-galactose and L-arabinose in the proportion of 6 to 1.[93, 94]

[89] See: W. L. Faith, *Ind. Eng. Chem.*, **37**, 9 (1945).

[90] E. Simonsen, Ger Patent 92,079 (1894); *Z. angew. Chem.*, **9**, 195, 962, 1007 (1896).

[91] A. Classen, Ger. Patent 118,540 (1899); 130,980 (1901)

[92] E. C. Sherrard and F. W. Kressman, *Ind. Eng Chem.*, **37**, 5 (1945).

[93] See. F. C. Peterson, A J. Barry, H. Unkauf and L E. Wise, *J Am. Chem. Soc.*, **62**, 2361 (1940).

[94] E. V. White, *J. Am. Chem. Soc.*, **64**, 2838 (1942); L. E. Wise and F. C. Peterson, *Ind. Eng. Chem.*, **22**, 362 (1930).

Alcoholysis of the methylated arabo-galactan yields the glycosides of 2,4-dimethylgalactose, 2,3,4-trimethylgalactose, 2,3,4,6-tetramethylgalactose and 2,3,5-trimethylarabinose in the relative proportions, 3:1:2:1. As a result of partial acid methanolysis, two products were isolated in addition to the previously obtained methylated glycosides. These products were tentatively identified as an octamethyl-(6-galactose D-galactoside) and a heptamethyl-(6-galactose D-galactoside) which has the unsubstituted hydroxyl at carbon 3 of the aglycon group. The composition of these disaccharides provides evidence that at least one of the polymeric linkages is of the 1,6' type, and the free hydroxyl at carbon 3 makes it probable that arabinose or galactose units are connected to this carbon in the intact polysaccharide. Inasmuch as the tetramethylgalactoside and the trimethylarabinoside fractions represent terminal groups, three of every seven units are end-groups and the molecule must be highly branched.

The allocation of the arabinose groups to terminal positions is supported by their preferential removal through partial hydrolysis of the arabogalactan.[95] The resulting product, after methylation and hydrolysis, consists of *equimolecular* quantities of di-, tri- and tetramethylgalactose residues. When the relative proportion of these products is compared with that obtained by the methylation and hydrolysis of the original arabogalactan, it will be seen that the arabinose components must be attached to galactose components which yield dimethylgalactose when the hydrolysis takes place after methylation and trimethylgalactose when there is a previous partial hydrolysis. However, the additional mole of trimethylgalactose, formed as a result of the preliminary removal of the arabinose groups, is the 2,4,6 isomer instead of additional 2,3,4 isomer. The arabinofuranose unit, then, must be attached to carbon 6 of the adjoining galactose residue in the original polysaccharide.

During methanolysis of the methylated polysaccharide, it appears that a resistant residue composed of a chain of 1,6' linked 2,4-dimethylgalactose residues accumulates in the early stages of the hydrolysis. If, as seems probable, this residue represents the main chain of the arabo-galactan, the probable structure may be written[95a]:

$$
\begin{array}{lll}
6'-1 & 6-1' & \text{(main chain)} \longleftrightarrow \\
\text{galactopyranose} & - & - \\
\mid 3 & & \\[2em]
1' & 6'-1 & \\
\text{(galactopyranose} & \text{galactopyranose)}_x \\
\mid 3' & & \\[1em]
\mid 1 & 6-1' & \\
\text{galactopyranose} & \text{arabinofuranose} &
\end{array}
$$

(Repeating unit of arabo-galactan according to White)
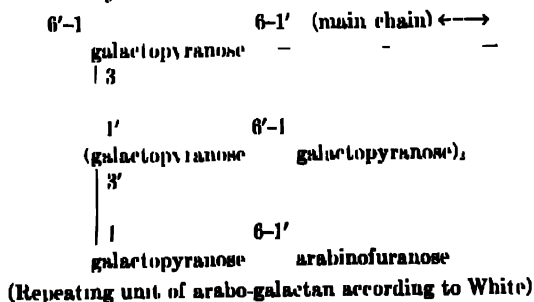
[95a] W. Low and E. V. White, *J. Am. Chem. Soc.*, **65**, 2430 (1944)

A highly branched structure agrees with the low viscosities exhibited by solutions of the polysaccharide. Since osmometric and viscometric measurements give values of about 220 for the degree of polymerization, the above unit structure occurs 31 times per molecule.[62]

As indicated above, there is some evidence that the arabo-galactan is heterogeneous. The acetylated and methylated material can be fractionated into materials with different optical rotations and pentose contents.[93, 95b] The ultracentrifuge shows that two fairly homogeneous components are present with molecular weights of 16,000 and 100,000.[95c]

Black spruce wood (*Picea mariana*) contains a water-soluble polysaccharide composed of D-galactose, L-arabinose and uronic acid in the following proportions: 4:1:1.[95d]

B. Mucilages, Gums and Gel-forming Substances.[75a, e, 95e] A group of substances which give viscous, mucilaginous or gelatinous solutions are variously described as plant gums, mucilages and gel-forming substances. In a very general way, it may be said that gums occur as exudations on the fruit and bark of plants. The exudations are frequently the result of insect produced injuries and possibly are the result of bacterial action. The extraction of plant seeds gives rise to mucilages and of marine algae (sea weeds) to the gel-forming carbohydrates. Until much more is known about these substances, it is impossible to characterize them more closely on structural considerations or physical properties. In general, uronic acids are present in the molecules as well as hexose and pentose sugars. An important distinction from the hemicelluloses appears to be the presence of free carboxyl groups which, for the hemicelluloses, are involved in linkages with some of the cell constituents (lignin ?). Frequently, the pentose constituents may be removed by "auto-hydrolysis" (i.e., by heating the gum in water) or by partial acid hydrolysis, and resistant residues composed of hexoses and uronic acids are left. The component sugars are found with pyranose rings except for L-arabinose which is encountered in the furanose form. Although the glucose polymers are usually formed through 1,4' glucosidic connections, galactose polymers frequently are found with 1,3' or 1,6' linkages. In spite of the wide distribution of gums, mucilaginous and gelatinizing substances, relatively few have received much investigation, and of these only several of the more important are discussed in the following pages.

The general composition of some plant gums, as summarized by Hirst,[75a] is given in Table IV.

[95b] E. L. Hirst, J. K. N. Jones and W. G. Campbell, *Nature*, **147**, 25 (1941).

[95c] H. Mosimann and The Svedberg, *Kolloid-Z.*, **100**, 99 (1942).

[95d] F. E. Brauns, *Science*, **102**, 155 (1945).

[95e] C. L. Mantell, "The Water Soluble Gums;" Reinhold, New York (1947).

Chemical tests have been devised for the identification of some of the plant gums and similar substances.[96]

The polysaccharides of seaweeds include gel-forming materials such as agar and carrageen mucilage (called geloses by Tseng) and nongelling materials such as alginic acid (a polyuronide) and laminarin (a poly glucoside with 1,3' linkages). Although the red and brown seaweeds have received some study, very little is known of the polysaccharides of the green and blue-green seaweeds. Tseng has proposed the name of phyco-colloids for the polysaccharides derived from brown and red seaweeds and capable of forming colloidal systems when dispersed in water.[97] In Fig. 3, a

TABLE IV

Approximate Composition of Some Plant Gums

(Moles of Constituent per Repeating Unit)

Gum	D Glucuronic Acid or Methoxyl Derivative	D-Galactose	D Mannose	L Arabinose	D Xylose	L-Rhamnose
Arabic	1	3	nil	2	nil	1
Damson	1	2	1	3	ca. 3%	nil
Cherry	1	2	1	6	ca. 3%	nil
Egg plum	2	6	nil	7	1	nil
Purple plum	1	3	nil	3(?)	1 (?)	nil
Almond tree	1	3	nil	4	2	nil
Lemon	+	+	nil	+	nil	nil
Orange	+	+	nil	+	nil	nil
Grape fruit	+	+	nil	+	nil	nil
Cholla	+	+	nil	+	nil	+
Mesquite	D-Galacturonic acid	+	nil	+	nil	—

tative arrangement of seaweed polysaccharides is given as proposed by Tseng. Some of these materials are discussed in more detail later.

Gum Arabic.[98] This water-soluble gum, found on the bark of members of the *Acacia* species, particularly of *A. Senegal* Willd, has appreciable industrial importance as an adhesive, size, thickener of liquids and stabilizer of emulsions. Medicinal uses include its application to soothe irritated tissues (demulcent action) and its substitution for blood transfusion in the treatment of surgical shock. Interestingly enough, the gum is produced only in hot, dry, elevated locations. Although the trees flourish under other

[96] M. B. Jacobs and L. Jaffe, *Ind. Eng. Chem*, Anal. Ed., **3**, 210 (1931).

[97] C. K. Tseng, *Science*, **101**, 597 (1945).

[98] "U. S. Dispensatory," p. 1; (22nd Ed.) J B Lippincott Co., Philadelphia (1937).
C. F. Mason, *Chem. Ind.*, **53**, 680 (1943).

SEAWEEDS (MARINE ALGAE)

GREEN SEAWEEDS (CHLOROPHYCEAE)   BROWN SEAWEEDS (PHAEOPHYCEAE)   RED SEAWEEDS (RHODOPHYCEAE)   BLUE-GREEN SEAWEEDS (CYANOPHYCEAE)

KELPS   GIANT KELPS   OTHERS   GULFWEEDS   ROCKWEEDS   AGAROPHYTES   CARRAGEENS   OTHERS
LEAF KELPS

Taxonomic groups

Sources: FUCOIDS

Seaweed genera: LAMINARIA MACROCYSTIS SARGASSUM FUCUS GRACILARIA GELIDIUM AHNFELTIA GIGARTINA CHONDRUS PHYLLOPHORA RHODOPHYCEA CYANOPHYTA

GELIDIUM AGAR   PTEROCLADIA AGAR   AHNFELTIA AGAR
GRACILARIA AGAR [AUSTRALIA S AFRICA N CAROLINA] [CALIFORNIA JAPAN CHINA] [NEW ZEALAND] [SAKHALIN SIBERIA WHITE SEA]

Phycocolloids: LAMINARIN   ALGIN   FUCOIDIN   AGAR (GELOSE)   CARRAGEENIN   AGAROID   RHODOPHYCIN   PURPURIN

Acids: ALGINIC ACID   AGARINIC ACID   CARRAGEENIC ACID   RHODOPHYCINIC ACID

Salts: SODIUM ALGINATE AMMONIUM ALGINATE CALCIUM ALGINATE CHROMIUM ALGINATE   SODIUM AGARINATE POTASSIUM AGARINATE CALCIUM AGARINATE MAGNESIUM AGARINATE   POTASSIUM CARRAGEENATE CALCIUM CARRAGEENATE   SODIUM RHODOPHYCINATE

Fig 3 Classification of some marine polysaccharides. After Tseng.

conditions, it is stated that no gum is produced. The commercial gum may contain material from other plants, and its widespread origin is indicated by some of the many synonyms used: African gum, galam gum, Kordofan or Arabian gum, Senegal gum, Sudan gum, hashab gum, etc.

Arabic acid is prepared from the natural gum, which occurs as the calcium salt, by treatment with acid and precipitation from solution as a result of the addition of alcohol. As determined by titration, the equivalent weight lies between 1,000 and 1,200. Complete acid hydrolysis produces galactose (29.5%), L-arabinose (34.4%), L-rhamnose (14.2%) and an aldobiuronic acid (28.3%) composed of galactose and glucuronic acid.[99] The analysis corresponds to 2 moles of galactose, 3 of arabinose, 1 of rhamnose and 1 of the aldobiuronic acid, approximately. Since Norman found no rhamnose in certain samples of gum arabic, it would appear that the composition may vary.

Mild acid hydrolysis removes the arabinose and rhamnose units as well as a disaccharide, 3-L-arabinose D-galactoside, and leaves a resistant degraded pectic acid-like substance composed of galactose and glucuronic acid in the proportion of 3 to 1. The composition of this degraded gum is demonstrated by the formation of 2,3,4,6-tetramethylgalactose, 2,3,4-trimethylgalactose, 2,4-dimethylgalactose and 2,3,4-trimethylglucuronic acid by the hydrolysis of the methylated derivative. Since the relative molecular proportions of the four products are, respectively, 1,5,3 and 3, the basic repeating unit consists of nine galactose and three glucuronic acid units. With the probable assumption of pyranose rings, the linkages must be of the 1,3' and 1,6' types. The large proportion of end groups necessitates a branched structure with three glucuronic acid residues and one galactose residue in the four terminal positions. Partial hydrolysis of methylated, degraded arabic acid leads to the isolation of 2,3,4,2',3',4'-hexamethyl-(6-galactose glucuronide); the degraded pectic acid gives 3-galactose galactoside. The aldobiuronic acid must represent a terminal grouping because of the lack of free hydroxyl groups. If all the uronic acid constituents are similarly connected, the aldobiuronic acids must represent detached side chains. The degraded arabic acid could then be represented as a continuous chain of galactose residues with 1,3' or 1,6' connections and with aldobiuronic acid side chains as represented on page 634.[100]

The structure of the undegraded arabic acid remains largely unknown although the degraded product probably represents an important basal unit. After direct methylation of arabic acid and subsequent hydrolysis, methylated L-rhamnopyranoside, L-arabinofuranosides and 3-arabinofuranose galactopyranoside are found among the products of hydrolysis. The isola-

[99] C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, **51**, 1519 (1929).

[100] J. Jackson and F. Smith. *J. Chem. Soc.*, **74**, 79, (1940).

```
   1-3'          1' 6        1-3'        1'-6        1-3'
galactose- - galactose -  galactose· —galactose- --galactose——galactose
            6'                         | 6'                  | 6'

             1                          | 1                   ｜1
          galactose                  galactose             galactose
                                        | 6                    6

             1'                          1'                  | 1'
          glucuronic                glucuronic            glucuronic
            acid                      acid                   acid
```
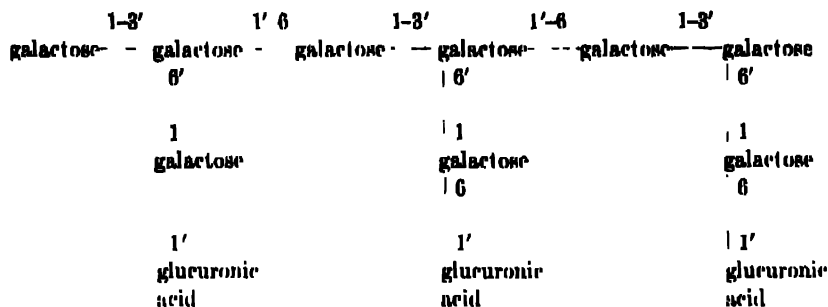
(Possible structure of degraded arabic acid)
(1,3' linkages may be 1,6' and 1,6' may be 1 3')

tion of methyl 2,3-dimethylglucuronide from these hydrolytic products indicates that the uronic acid residues of the undegraded alginic acid may not always act as end groups; instead, the side chains may be longer, or groups other than galactose units may be found in the main chains. The complexity of the products of hydrolysis, however, make the isolation and quantitative measurement of the components an extremely difficult problem.[101]

Mesquite Gum. This gum, which provides an excellent source of L-arabinose, exudes from the stems and branches of small thorny shrubs or trees (*Prosopis juliflora* DC) growing in the southwestern United States. In general properties and composition, it resembles gum arabic. The natural product, a salt, is converted to an acid by acidification of gum solutions and precipitation of the gum acid with alcohol. Analysis of the acid reveals the following constituents and proportions: L-arabinose (4 moles), D-galactose (3 moles) and methylglucuronic acid (1 mole). By mild acid hydrolysis, the L-arabinose portion of the gum is selectively removed, and a resistant residue composed of galactose and uronic acid units remains.[102]

According to analyses by White,[104] the composition of the gum is better expressed by the ratio: 4:2:1 for arabinose:galactose:uronic acid. Methylation studies on the unpurified gum show that seven residues are present in a repeating unit. Of these, one methyl uronic acid and one arabinose unit occupy end positions. The remaining arabinose units have 1,2 linkages, whereas the galactose units are substituted in both the 3 and 6 positions. Hence, the material is highly branched.

Gum Ghatti. Gum ghatti (Indian gum) from an Indian tree (*Anogeissus latifolia*, Wall) appears to be similar in composition to mesquite gum although the uronic acid component has not been positively identified. The

[101] F. Smith, *J. Chem. Soc.*, 1035 (1940).
[102] E. Anderson and L. Otis, *J. Am. Chem. Soc.*, **52**, 4461 (1930).
[104] E. V. White, *J. Am. Chem. Soc.*, **68**, 272 (1946).

molecular weight determined osmometrically is reported to be 11,860.[104] Gum ghatti finds application in pharmacy for the same uses as gum arabic.

Gum Tragacanth. A gum exudation produced by shrubs of the genus *Astragalus* (order, *Leguminosae*) and found in the Middle East and southwestern Europe is known as gum tragacanth. The gum is very heterogeneous in composition.[105] It contains an acidic carbohydrate material (tragacanthic acid), a neutral polysaccharide and probably a glycoside.[105b]

Alcoholysis of the methylated tragacanthic acid and fractionation give: methyl 2,3,4-trimethyl-α-L-fucoside (I), methyl 2,3,4-trimethyl-D-xyloside (II), methyl 3,4-dimethyl-D-xyloside (III), methyl ester of methyl 2,3-dimethylgalactofururonide (IV), and methyl ester of a methyl monomethyl-galacturonide (V). The fucose (I) and xylose (II) residues represent terminal groups since they are fully substituted. Some of the xylose residues (III) must be involved in 1,2'-linkages, whereas the galacturonic acid residues may have 1,5', 1,6' or more probably 1,4' linkages.

The neutral polysaccharide contains L-arabinose and D-galactose.[107] Methylation studies show that it must be highly branched and that the arabinose residues have a furanose structure.

Carob Bean Gum. The bean of *Ceratonia siliqua* L., which is found in regions adjoining the Mediterranean, is used as a food under the name of St. John's bread. It yields a gum, called locust bean gum, carob bean gum or caroubine, which has considerable commercial value as a size for textiles, and as a component of mucilages, pharmaceuticals, cosmetics, etc. Subsequent to the early investigations by Effront and other workers, the gum was shown to consist of mannose and galactose residues.[108] The composition of this material has been extensively studied by Lew and Gortner.[109]

Mannose (determined as the hydrazone) and galactose (determined by oxidation to mucic acid) were found in the hydrolyzates in the proportions of 3 to 1. Uronic acids, pentoses, ketoses and glucose could not be identified in the products of hydrolysis and seem to be absent. When the gum is treated with 0.2 N hydrochloric acid at 100°C., the galactose components are selectively removed, and a residue is left which contains only mannose units. This evidence may be interpreted as indicating that the gum is a heterogeneous mixture of a mannan and a galactan or that the basic chain consists of a series of mannose residues connected to a series of galactose residues.

[104] D. Hanna and E. H. Shaw, Jr., *Chem. Abst.*, *36*, 3386 (1942)

[105] See: C. O'Sullivan, *J. Chem. Soc.*, *79*, 1164 (1901).

[105b] S. P. James and F. Smith, *J. Chem. Soc.*, *739*, 746 (1945). These articles also discuss the earlier work.

[107] S. P. James and F. Smith, *J. Chem Soc* 749 (1945).

[108] E. Bourquelot and H. Hérissey, *Compt. rend.*, *129*, 228, 391 (1899).

[109] B. W. Lew and R. A. Gortner, *Arch. Biochem.*, *1*, 325 (1943).

During oxidation of the gum with periodic acid, one mole of oxidant is consumed per hexose unit. Only 1,4' or 1,2' linkages are compatible with this result. A 1,6' linkage would result in two moles of oxidant being used, and a 1,3' linkage would require that no oxidation take place since no unsubstituted glycol groups could be present. Inasmuch as the aldehydes produced by the action of periodic acid are oxidized to D-glyceric and tartronic acid, the principal polymeric linkage must be of the 1,2' type. The direction of mutarotation of the products of hydrolysis of the gum and of the residual mannan indicate that the galactoside connections have the alpha configuration and the mannoside connections the beta configuration.

A material having a composition similar to that of carob bean gum occurs in the endosperm of seeds from an Indian forage plant known as guar (*Cyamopsis proraloides* or *C. tetragona*). The material shows considerable promise as a beater size in paper manufacture.[110]

Endosperm mucilages from the following plants contain manno-galactans[111]:

Source	% Galactan
Guar (*Cyamopsis* sp.)	34–34
Locust bean (*Ceratonia siliqua*)	20
Honey locust (*Gleditsia triancanthos*)	26
Flame tree (*Delonix regia*)	18–19
Kentucky coffee bean (*Gymnocladus dioica*)	25–27
Palo verde (*Cercidium torreyanum*)	21–22
Tara (*Caesalpinia spinosa*)	26
Huizache (*Caesalpinia cacalaca*)	27
Sophora japonica	16

The remainder of these mucilages consists essentially of mannose residues.

Agar. "Agar is the dried mucilaginous substance extracted from *Gelidium corneum* (Hudson) Lamouroux and other species of *Gelidium* (Fam *Gelidiaceae*) and closely related Algae (Class *Rhodophyceae*)[112]" (see Fig. 3).

Many sea weeds growing along the coasts of the Carolinas, Florida, California and southern Asia contain an extractable carbohydrate known under a variety of names: agar, agar-agar, vegetable gelatin or glue, Japanese isinglass, etc. In order to obtain the material, the sun-dried sea weeds are boiled with water, and the extracts are filtered through coarse cloths and poured into shallow pans to cool and solidify. The property of agar of forming gelatinous solutions is widely utilized in the preparation of semi-solid cultural media for growing bacteria, as a component of emulsions

[110] B. W. Rowland, *Chemurgic Digest*, **4**, 369 (1945).

[111] L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, **16**, 26 (1944).

[112] "U. S. Dispensatory," p. 88 (22nd Ed.); J. B. Lippincott Co., Philadelphia (1937), see also: C. K. Tseng, *Scientific Monthly*, **58**, 24 (1944).

(including photographic emulsions), in foodstuffs as a thickener, and in adhesives and sizes. Its use as a cathartic depends upon its indigestibility and the swelling which takes place in the presence of water. By virtue of these properties it passes into the intestines where swelling takes place, and the bulk of the intestinal contents increases.

In addition to D-galactose, the hydrolysis products include the enantiomorphous L-galactose and sulfuric acid. Agar apparently exists as the calcium or magnesium salt of the sulfuric acid ester of a polysaccharide consisting of D- and L-galactose units.[113]

Agar may be acetylated by the pyridine-acetic anhydride method. The acetate on methylation and hydrolysis yields 2,4,6-trimethyl-D-galactose, the unique 2-methyl-3,6-anhydro-L-galactose and no tetramethylgalactose.[114] From the well known tendency of sulfate esters of the sugars to yield anhydro rings on hydrolysis, it would seem probable that the anhydro derivative is a secondary product due to removal of the sulfate group during methylation. This origin of the anhydro derivative receives support from the formation of 3,6-anhydrogalactose from methyl galactoside 6-sulfate as a result of alkaline hydrolysis.[115] Acid hydrolysis of the galactoside, however, proceeds without anhydro formation. Percival and Thomson believe the anhydro-L-galactose may be a constituent of agar since its quantity is larger than can be accounted for by the sulfur content of the agar used. The presence of blocking groups in all three positions of agar is supported by the complete resistance of the substance to the action of periodic acid.[116] If position 3 as well as 2 were unsubstituted, the glycol group would be oxidized.

The most probable structure for agar, based on the evidence cited, involves a chain of D-galactopyranose units connected through 1,3′ linkages and with a single L-galactopyranose unit at one end.[114] The L-galactose residue is connected through the hydroxyl of carbon 4 with the first carbon of the adjacent D-galactopyranose unit and carries a sulfate group at carbon 6.

	1–3′		1′–3		1–4′	
D galactose		(D galactose)$_n$		D-galactose		L-galactose 6-sulfate

(Basic repeating group of agar)
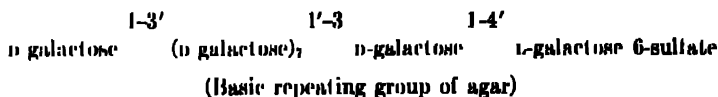
[113] See W. F. Hoffman and R. A. Gortner, *J. Biol. Chem.*, **65**, 371 (1925); N. W. Pirie, *Biochem. J.*, **30**, 369 (1936)

[114] I. A. Forbes and E. G. V. Percival, *J. Chem. Soc.*, 1846 (1939); W. G. M. Jones and S. Peat, *ibid.*, 225 (1942).

[115] E. G. V. Percival and T. G. H. Thomson, *J. Chem. Soc.*, 750 (1942); E. G. V. Percival, *Nature*, **154**, 673 (1944)

[116] V. C. Barry, T. Dillon and W. McGettrick, *J. Chem. Soc.*, 183 (1942); V. C. Barry and T. Dillon, *Chemistry & Industry*, 167 (1944).

Jones and Peat considered that the repeating unit contains ten hexose units. Barry and Dillon found much less sulfur than is required by the Jones and Peat formula and believe that the complete resistance to periodic acid may be explained by the presence of some 3,6-anhydro linkages in the units connected by 1,4' bonds; they found that the agar chains have a minimum length of 140 units, which result corresponds to the sensitivity of the oxidation method.

Although the presence of a molecule of L-galactose in the repeating unit seems strange, it is not difficult to devise a biological genesis. The symmetry of the galactose molecule is such that a D-galactose 1-sulfate (I) linked through carbon 3 to another galactose molecule could be transformed by a simple oxidation-reduction reaction to the L-galactose 6-sulfate (II) substituted at carbon 4.

(I)   (II)

(Aldehyde form)
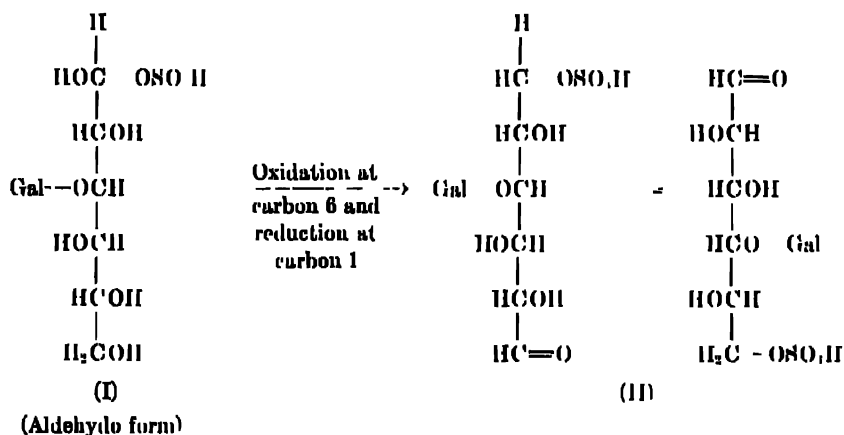
Other polysaccharide sulfate esters have been described. Carrageen mucilage,[117] extractable from Irish moss (*Chondrus crispus*, a marine alga), contains considerable quantities of the potassium and calcium salts of a sulfate ester and at least 30% consists of a mixture of two galactans. The galactan linkage is 1,3', and the sulfate groups are at C-4. 2-Ketogluconic acid has been isolated from the hydrolysis products.

Sea weed polysaccharides from *Gigartina stellata* (used in the preparation of "British Agar") and from *Dilsea edulis* appear to have many 1,3' galactosidic linkages and also sulfate groups at carbon 4.[118]

[117] P. Haas, *Biochem. J.*, *15*, 469 (1921); M. R. Butler, *ibid.*, *28*, 759 (1934); T. Dillon and P. O'Colla, *Nature*, *145*, 749 (1940); E. G. Young and F. A. H. Rice, *J. Biol. Chem.*, *156*, 781 (1944); J. Buchannan, E. E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 51 (1943).

[118] E. T. Dewar and E. G. V. Percival, *Nature*, *156*, 633 (1945); V. C. Barry and T. Dillon, *Proc. Roy. Irish Acad.*, *50B*, 349 (1945); *Chem. Abst.*, *40*, 3728 (1946).

Iridophycin, a galactan sulfate from the marine alga *Iridea laminarioides*, may consist of a chain of galactopyranose residues connected through the 1,4′ positions with each residue esterified at position 6 by a molecule of sulfuric acid.[119]

Several polysaccharides other than agar also contain L-galactose as a constituent. Anderson[120] notes its presence among the hydrolysis products of linseed mucilage and suggests this mucilage as a source for the sugar. Snail galactogen, previously discussed, yields the sugar on hydrolysis.

<div align="center">TABLE V</div>

Classification of Polysaccharides Found Associated with Proteins

I. Mucopolysaccharides.
    A. Having uronic acids as a constituent.
        1. Not esterified with sulfuric acid.
            a. Polysaccharides of vitreous humor, umbilical cord, synovial fluid, group-A hemolytic streptococcus.
            b. Some bacterial polysaccharides (for discussion see under B, 2).
        2. Esterified with sulfuric acid.
            a. Chondroitin sulfuric acid.
            b. Mucoitin sulfuric acid from cornea and gastric mucin.
            c. Heparin.
    B. Neutral mucopolysaccharides.
        1. Chitin
        2. Many bacterial polysaccharides.
        3. Blood and gastric polysaccharides.
II. Glycoproteins.
    A. Ovomucoid-α (ovomucoid).
    B. Ovomucoid-β (ovomucin).
    C. Serum mucoid, serum glycoid.
    D. Egg white, serum and thyroglobulins.
    E. Pregnancy urine hormone.

C. Polysaccharides Associated with Proteins and/or Microorganisms.[121] [122] [123] This group of polysaccharides is of great biological importance, but because of their complexity they have not received enough investigation, except in a few instances, to make it possible to present very definite structures. With the principal exception of the bacterial levans, dextrans, etc. and of chitin, polysaccharides of the group under considera-

[119] W. Z. Hassid, *J. Am. Chem. Soc.*, **57**, 2046 (1935).

[120] E. Anderson, *J. Biol. Chem.*, **100**, 249 (1933)

[121] K. Meyer, *Cold Spring Harbor Symposia Quant. Biol.*, **6**, 91 (1938).

[122] A. G. Norman, *loc. cit.*[78] p. 197, T. H. Evans and H. Hibbert, *Advances in Carbohydrate Chem.*, **2**, 203 (1946).

[123] M. Stacey, *Chemistry & Industry*, **62**, 110 (1943); *Advances in Carbohydrate Chem.*, **2**, 161 (1946).

tion usually yield several types of monosaccharides on hydrolysis and frequently also amino sugars and uronic acids A tentative scheme for the classification of these polysaccharides, presented by K. Meyer, is reproduced above (Table V) with several modifications and is followed in the subsequent discussion. M. Stacey has presented a similar but more detailed scheme. Two main subdivisions are established: the mucopolysaccharides, which occur in nature as free polysaccharides or as easily dissociable salts of proteins; the glycoproteins, which have the polysaccharides combined with proteins through stable (covalent?) linkages.

A method for the qualitative and quantitative estimation of the monosaccharide constituents depends on the reaction of orcinol or carbazole with the products of hydrolysis by strong sulfuric acid solutions.[124] Colored products are formed which by photometric procedures can be used for the quantitative and qualitative determination.

D. Mucopolysaccharides. *Mucopolysaccharides Containing Uronic Acids and Free of Sulfur.* A substance called hyaluronic acid has been isolated from vitreous humor, umbilical cord, synovial fluid, Group A hemolytic streptococcus and certain tumors.[125] On hydrolysis it gives equimolecular proportions of glucosamine, glucuronic acid and acetic acid. An enzyme, occurring in hemolytic streptococcus and in the uveal tract of the eye, hydrolyzes the polysaccharide to glucuronic acid and N acetylglucosamine.[1] It seems to be similar or identical to Dubos' "autolytic enzyme of pneumococcus" which autolyzes all types of pneumococci but does not act on the type-specific polysaccharides. A substance similar to hyaluronic acid, therefore, is probably to be found in pneumococci of all types.[1] Proteins with basic amino groups form salts with hyaluronic acid, and these are precipitated at acidities greater than those at the isoelectric points of the proteins alone. The occurrence of this polysaccharide in synovial fluid, vitreous humor and hemolytic streptococcus is of interest since inflammatory conditions in joints and in the eyes are often associated with streptococcus infections (e.g., rheumatic fever).[121]

The sulfuric acid ester of hyaluronic acid has been isolated from cattle corneas.[126]

Mucopolysaccharides Containing Uronic Acids and Esterified with Sulfuric Acid. The most important members of this group are chondroitin sulfuric acid, mucoitin sulfuric acid and heparin. Chondroitin sulfuric acid, isolated from cartilage, yields on hydrolysis approximately equimolecular

---

[124] M. Sørensen and G. Haugaard, *Biochem. Z.*, 260, 247 (1933); S. Gurin and D. B. Hood, *J. Biol. Chem*, 139, 775 (1941).
[125] K. Meyer, E. M. Smyth and M. H. Dawson, *Science*, 88, 129 (1938); F. E. Kendall, M. Heidelberger and M. Dawson, *J. Biol. Chem*, 118, 61 (1937); E. A. Kabat, *ibid.*, 130, 143 (1939).
[126] K. Meyer and E. Chaffee, *Am J. Ophthalmol.*, 23, 1320 (1940).

quantities of 2-desoxy-2-aminogalactose (chondrosamine), glucuronic acid, sulfuric acid and acetic acid.[126a] Partial hydrolysis gives a nitrogenous aldobiuronic acid, chondrosin, for which Levene has suggested the following formula:
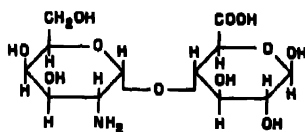
Neither the position of the galactosidic linkage nor its configuration has been determined. Molecular weight determinations (cryoscopic and diffusion methods) have given uncertain values, but the molecule probably contains two aldobiuronic acid units.[127] A methylated degraded chondroitin of low molecular weight and free of sulfate groups has been prepared.[128] Acid methanolysis produces derivatives of glucuronic acid and 2-desoxy-2-aminogalactose; among the products are methyl 2,3,4-trimethyl-α-glucuronide and methyl 3,4,6-trimethyl-2-desoxy-2-acetylaminogalactoside. Hence, the repeating unit probably has a branched structure such as:
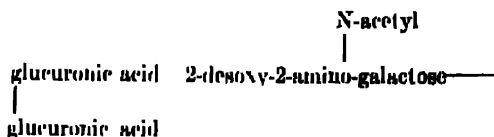
When neutralized solutions of chondroitin sulfuric acid are mixed with solutions of proteins (e.g., gelatin, egg albumen) and acid is added, precipitation takes place. Titration of the precipitate shows that both acid groups of the chondroitin sulfuric acid are combined with basic groups of the proteins. Frequently, these complexes may be broken up into protein and acid by the addition of inorganic salts.

A polysaccharide, similar to chondroitin sulfuric acid but containing 2-desoxy-2-aminoglucose (glucosamine), is found in gastric mucosa and cornea and possibly in other sources and is called mucoitin sulfuric acid.[121, 126a] The proportion of sulfuric acid present depends on the process used; alkaline procedures give products with about one-half mole of sulfuric acid, and neutral procedures products with about one mole of the acid per aldobiuronic acid unit.

[126a] P. A. Levene, "Hexosamines and Mucoproteins;" Longmans, Green & Co., London (1925); P. A. Levene, J. Biol Chem., 140, 267 (1941); K. Meyer and E. M. Smyth, J. Biol. Chem., 119, 507 (1937).

[127] O. Fürth and T. Bruno, Biochem. Z., 294, 153 (1937).

[128] H. G. Bray, J. E. Gregory and M. Stacey, Biochem. J., 38, 142 (1944).

Heparin.[129] An important polysaccharide derivative called heparin, found widely distributed in animal tissues, is an effective inhibitor of blood coagulation. Although the structure has not been well established, heparin appears to be the sulfate ester of a polysaccharide which consists of acetylated glucosamine and glucuronic acid residues. It has been claimed that the sulfate groups are mainly responsible for the anticoagulant properties, for sulfate esters of other polysaccharides and organic compounds exhibit similar but less pronounced action. Polysulfates of chondroitin and cellulose have relatively strong anticoagulant activity which however is less than that of heparin; the compounds also are more toxic.[130]

Wolfrom and McNeely,[131] however, have shown that one of the nitrogen atoms of heparin is particularly significant. Thus, mild acid hydrolysis inactivates heparin at the same rate as amino groups (by Van Slyke analysis) are produced.

The sodium salt is recommended as a substitute for citrate in transfusions.

Neutral Mucopolysaccharides. The first member of this group, chitin, has been discussed earlier in this chapter.

E. Bacterial and Fungal Polysaccharides. Polysaccharides produced by microorganisms are found as soluble or insoluble products (gums) in the culture solution, as capsules surrounding the organisms, or as reserve or structural constituents of the cells. Many are of particular interest as they possess immunological properties. Although some are homopolysaccharides and are considered in detail earlier, general properties of the group will be considered in the present section. For this reason, those containing uronic acids are also included here. Table VI taken from Norman[132] [133] summarizes some of the products formed by the "gum"- or "slime"-producing bacteria. For the yeast polysaccharides, see the discussion under dextrans and mannans.

The organisms often are responsible for the "slimes" formed in carbohydrate solutions; the "slimes" or "gums" are mainly of carbohydrate nature. Some of these organisms are very selective in their action, but others are much less specific. The levan formers apparently require fructose as a substrate or a di- or oligosaccharide containing this sugar in a terminal position. Thus, sucrose (1-glucose fructofuranoside) and raffinose (galactose-glucose-fructose) are utilized by the organisms while melezitose (glucose-fructose-glucose) is not. Even more marked specificity is shown by

[129] J. E Jorpes, *Z. physiol. Chem.,* *278*, 7 (1943); E. Chargaff and K. B. Olson, *J. Biol Chem.,* *128*, 153 (1937); W. H. Howell, *Am. J. Physiol.,* *9*, 1, (1912).

[130] P. Karrer, H. Koenig and E. Usteri, *Helv. Chim. Acta,* *26*, 1296 (1943).

[131] M. L. Wolfrom and W. H. McNeely. *J. Am. Chem Soc.,* *67*, 748 (1945); M. L. Wolfrom and F. A. H. Rice, *ibid.,* *68*, 532 (1946).

[132] A. G. Norman, *loc. cit.,*[7b] p. 197.

[133] See also: F. C. Harrison, H. L. A. Tarr and H. Hibbert, *Can. J. Research,* *3*, 449 (1930).

Leuconostoc species which synthesize dextrans from sucrose but not from glucose.[133, 134] Other organisms are not nearly so specific and form mannans and galactans from glucose, etc. The structures of several dextrans and levans are considered in more detail earlier in this chapter.

TABLE VI

List of "Gum-forming" Bacteria

(after Norman)

"Gum" Producing Bacteria	Gum Type
Clostridium gelatigenosum	Levan
Semiclostridium commune	"
Bacillus laevaniformans	"
" *lactis*	"
" *megatherium*	"
" *mesentericus*	"
" *subtilis*	"
" *vulgatus*	"
" *panis*	"
" *ruminatus*	"
Pseudomonas pruni	"
" *prunicola*	"
Bacterium eucalypti	"
Leuconostoc mesenteroides	Dextran
" *dextranicus*	"
Micrococcus viscosus	"
" *gelatinogenus*	"
Bacillus gummosus	"
" *panis viscosus*	"
Bacterium atherstonei	Galactan
" *sacchari*	"
Rhizobium limosospongiae	"
Bacterium acaciae	Arabo-galactan
" *metarabinum*	"
" *pararabinum*	"
Bacillus krzemieniewski	Mannan
Acetobacter xylinum	Cellulose
" *xylinoides*	"

An interesting polysaccharide from the mold *Penicillium luteum* is luteic acid which has been shown to be the half-ester of a polysaccharide "luteose" and malonic acid. Luteose consists of glucose units polymerized through 1,6'-linkages. Alkaline hydrolysis of luteic acid gives a molecule of malonic acid tor each two glucose units.[135]

[134] H. L. A. Tarr and H. Hibbert, *Can. J. Research*, **5**, 414 (1931).

[135] H. Raistrick and M. L. Rintoul, *Trans. Royal Soc. (London)*, **B220**, 255 (1931); C. G. Anderson, W. N. Haworth, H. Raistrick and M. Stacey, *Biochem. J.*, **33**, 272 (1939).

Immuno-polysaccharides. An important advance was recorded in carbohydrate and immuno-chemistry when it was reported by Dochez and Avery[134] that pneumococci form readily soluble substances which diffuse into the culture medium and which were identified by Heidelberger and Avery as polysaccharides responsible for the immunological specificity of these bacteria. These polysaccharides are found in the blood and urine of pneumonia patients. Subsequently many other bacteria were found to produce immunologically specific polysaccharides. For a more detailed discussion of immunological reactions particularly of the simpler synthetic substances, see Chapter IX. Some of these polysaccharides are antigenic and are capable of producing antisera in the animals into which they are injected. Others, however, act as haptenes and although they precipitate antisera evoked by the whole antigen, they are not complete antigens themselves.[137]

Many different strains of pneumococci are known. The various strains numbering at least 40 types, are differentiated on the basis of their immunological reactions.[139] The capsules of the bacteria and the culture solutions contain polysaccharides which have been shown to be the type-specific substances. The general composition and properties of the polysaccharides of 32 pneumococcus types have been investigated by R. Brown[140] Ten types are essentially free of nitrogen; a maximum nitrogen content of 4 to 5% is found in polysaccharides of types 1,4,5,12 and 25. Type 1 polysaccharide contains 2% amino-nitrogen, but the others contain very little if any nitrogen in this form. Thirteen of the 32 types have phosphorus associated with the polysaccharides. Types 28 and 32 contain as much as 6% phosphorus, and in 12 others the phosphorus content exceeds 3% Acetyl groups are also associated with the polysaccharides, and analyses show that the acetyl content lies between the value of 0.32% for type 3 and 16.33% for type 11. With the exception of type 23, all the polysaccharides are optically active, 25 being dextro-rotatory and 7 levo-rotatory Except for type 5, none reduces Fehling solution under mild conditions. Color tests for uronic acids are positive for types 1,2,3,8,9,12,22,25 and 27. Although the dimethylaminobenzaldehyde test for amino acids is negative for all of the polysaccharides, it is given after acid hydrolysis strongly by 17 types and weakly by 3 more. Homologous immune serums are precipitated by all of the polysaccharides in dilutions as high as 1:4,000,000.

In later work, the dextro-rotatory type 33 polysaccharide was found to

[134] A. R. Dochez and O. T. Avery, *J. Exptl. Med.*, *26*, 477 (1917); M. Heidelberger, and O. T. Avery, *ibid*, *38*, 73 (1923).

[137] H. Raistrick and W. W. C. Topley, *Brit. J. Exptl. Path*, *15*, 113 (1934).

[138] W. C. Boyd, "Fundamentals of Immunology;" Interscience, New York (1943)

[139] R. Brown, *J. Immunol*, *57*, 445 (1939); *Chem Abst.*, *36* 5884 (1942)

be a uronic acid derivative containing 1.65% nitrogen, 11.24% acetyl groups and no phosphorus.

The constitution and structures of the pneumococcal polysaccharides have been extensively investigated by Avery, Goebel, Heidelberger and associates.[140] The type 3 polysaccharide has received the most study. It has an unusual structure consisting of alternate glucopyranose and glucuronic acid residues. The glucosidic linkages are most easily hydrolyzed by acids so that a high yield of an aldobiuronic acid is obtained. Methylation studies[141] carried out with the polysaccharide and the disaccharide show that the polymeric linkages alternate between the 1,3' and 1',4 positions. The linkages probably have beta configurations. (The primed numbers refer to the glucuronic acid residues and the others to the glucose residues.)

$$
\underset{\beta}{\overset{1,3'}{\text{glucopyranose}}} - \underset{\beta}{\overset{1',4}{\text{glucuronic acid}}} \quad \underset{\beta}{\overset{1,3'}{\text{glucopyranose}}} \text{—glucuronic acid——}
$$
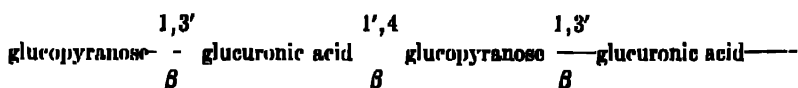
It has been shown that the heterophyle antigen of pneumococcus (type 1) is closely related in structure to the corresponding cellular polysaccharide. Although extremely resistant to hydrolysis, it yields after acid hydrolysis about 6% of a fatty acid of fairly high molecular weight and apparently the cellular polysaccharide.[142]

Other bacterial polysaccharides have also been investigated. That formed by *B. dysenteriae* yields after acid hydrolysis 97% of a reducing substance (calculated as glucose). It contains 1.7% nitrogen, 5% acetyl and has an acid equivalent of 9000. Since nitrogen is not liberated by the action of nitrous acid, the amino groups are probably acetylated. Galactose (15%), L-rhamnose (7.5%) and a N-acetylamino sugar (25%) are among the hydrolysis products.[143] Tubercle bacilli polysaccharides on hydrolysis give mannose and possibly L-arabinose and glucose in variable proportions.[144] Equivalent quantities of galactose and glucosamine are produced by the hydrolysis of the polysaccharide from the anthrax bacillus, and the two constituents make up 68% of the intact polysaccharide, which also contains

[140] M. Heidelberger, F. E. Kendall and H W. Scherp *J. Exptl. Med.*, *64*, 559 (1936).

[141] R. E. Reeves and W. F. Goebel, *J. Biol. Chem*, *139*, 511 (1941)

[142] W. F. Goebel, T. Shedlovsky, G. I. Lavin and M. H. Adams, *J. Biol. Chem.*, *148*, 1 (1943).

[143] W. T. J. Morgan, *Helv. Chim Acta*, *21*, 469 (1938). The serological specificity of various strains have been studied by: N. N. Spassky and L. A. Danenfeldt, *Bull. biol. méd. expll.*, *U.R.S.S.*, 7, 202 (1939)

[144] R. J. Anderson, R. L. Peck and M M. Creighton, *J. Biol. Chem*, *136*, 211 (1940); A. E. O. Menzel and M. Heidelberger, *ibid.*, *127*, 221 (1939).

acetyl groups.[145] A polysaccharide isolated from diphtheria bacteria contains bound galactose and probably D-arabinose and chondrosamine.[146]

The specific polysaccharide of type-B *Hemophilus influenzae* gives a positive test for uronic acids and has the following analysis: N, 0.3%; P, 8.54% (mostly organic); ash, 27.4%; and reducing power as glucose, 0.6%. With immune horse serums, specific precipitation occurs in dilutions as high as 1:35,000,000.[147]

The polysaccharide produced by the fungus *Coccidioides immitis* gives positive precipitative reactions with antisera. On acid hydrolysis, it yields glucose, galacturonic acid and unknown sugars.[148]

Blood Polysaccharides. Certain polysaccharides which have been isolated from the blood and urine are related to the blood types of the animals from which they are obtained.[149] The specific polysaccharides obtained from individuals belonging to groups II (A) and III (B) are active haptenes and combine with the α- and β-agglutinins of human blood serum. The monosaccharides formed after acid hydrolysis include N-acetylglucosamine, glucosamine, mannose and galactose. L-Fucose also has been identified. Since it is isolated as the trimethyl derivative, it appears to be present in the polysaccharide in a terminal position. Removal of the acetyl groups from the group-A polysaccharide results in a loss of haptene effect which is regained after reacetylation.

Capsular pneumococcus polysaccharides are sometimes found in the blood of pneumonia patients and their presence usually indicates a severe infection.[150] The blood group-A specific substance precipitates the antibody of type-14 antipneumococcus horse serum. Both the type-14 polysaccharide and the blood substance are composed of galactose and acetylglucosamine (in the ratio of 3:1), and the precipitation is probably the result of a close structural relationship between the two substances.[151] The presence of mannose could not be demonstrated. A neutral complex of a polysaccharide and an amino acid, apparently identical with the blood group-A substance, is found in commercial gastric "mucin."[121] [152]

F. Glycoproteins. The carbohydrate component of egg albumin is ob-

[145] G. Ivánovics, *Z. Immunitäts.*, **97**, 402 (1940).

[146] E. M. Gubarev, *Biokhimiya*, **7**, 180 (1942); *Chem. Abst.*, **38**, 142 (1944).

[147] J. H. Dingle and L. D. Fothergill, *J. Immunol.*, **37**, 53 (1939).

[148] W. Z. Hassid, E. E. Baker and R. M. McCready, *J. Biol. Chem.*, **149**, 303 (1943).

[149] A. V. Stepanov, A. M. Kuzin, Z. Maksaeva and P. Kosyakov, *Biokhimiya*, **5**, 547 (1940); H. Bierry, B. Gouzon and C. Magnan, *Compt. rend. soc. biol.*, **130**, 411 (1939); K. Freudenberg and H. Eichel, *Ann.*, **518**, 97 (1935); H. G. Bray, H. Henry and M. Stacey, *Biochem. J.*, **38** xxii (1944)

[150] S. C. Bukantz, J. G. M Bullowa and P de Gara, *Proc. Soc. Exptl. Biol. Med.*, **41**, 250 (1939).

[151] W. F. Goebel, P. B. Beeson and C. L. Hoagland, *J. Biol. Chem.*, **129**, 455 (1939).

[152] W. T. J. Morgan and H. K. King, *Biochem. J.*, **37**, 640 (1943).

tained by enzymic digestion of ovomucoid with trypsin. It is reported to have a molecular weight of about 1200. On hydrolysis, mannose, glucosamine, acetic acid and galactose are produced.[164] Stacey and Woolley, who have investigated the structure by the methylation method, report that the products of hydrolysis of the methylated polysaccharide are: N-acetyl-3,4,6-trimethyl-D-glucosamine (7 moles), D-mannose (2 moles), 3,4,6-trimethyl-D-mannopyranose (1 mole) and tetramethyl-D-galactopyranose (1 mole). The presence of free mannose indicates that the molecule is of an unusual type in which the mannose units occupy a central position and are completely substituted by glycosidically bonded sugars. The other substituents, except one mole of mannose, appear to occupy terminal positions.

The compositions of the other glycoproteins listed in K. Meyer's classification (p. 639) are still rather indefinite. The reader is referred to the original paper and to the review by Stacey[173] for further discussions of these compounds.

[164] A. Neuberger, *Biochem. J.*, *32*, 1435 (1938); P. A. Levene, *J. Biol. Chem.*, *140*, 279 (1941); M. Stacey and J. M. Woolley, *J. Chem. Soc.*, 184 (1940); 550 (1942).

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